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Premium Research Tools

2022

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about highQu

Since 2013, highQu assists life science research community worldwide by fueling it with molecular biology tools of premium quality. Being especially strong in providing robust and fast enzymes for PCR and qPCR enabling direct amplification of crude samples, we are also proud to offer unique solutions for other applications, such as non-toxic DNA gel stains or room-temperature stable protein and DNA electrophoresis ladders.

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NEW in 2022

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Isothermal RNA amplification, LAMP	**ALLin™ Isothermal 1Step RNA Amplification Kit**	Efficient isothermal RNA amplification in one step at elevated temperatures, robust performance on complex RNA templates.	10
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highQu professionally simple

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highQu enzymes and master mixes are well-known for their excellent performance and ease of use with minimum optimization required. Supplied with PCR water, optimized for the most robust performance, our kits provide reproducible results from sample to sample.

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Isothermal Amplification Enzymes and Kits

Selection of Isothermal Amplification Tools

Product	Key Applications	Enzymes	Template	3'-5' exo	5-3' exo	Optimal Temp.	Inact. Temp.
ALLin™ Isothermal DNA Amplification Kit	Isothermal DNA amplification at 70°C, LAMP	Bst DNA Polymerase large fragment	DNA			65°C	10 min. at 80°C
ALLin™ Isothermal 1Step RNA Amplification Kit	Virus RNA detection, SARS-CoV-2 detection, Isothermal RNA amplification, RT-LAMP	Reverse Transcriptase with RNase Inhibitor. Bst DNA Polymerase large fragment.	RNA			65°C	10 min. at 80°C
phi29 DNA Polymerase	Isothermal DNA amplification at 30°C, RCA, WGA, MDA	Recombinant phi29 DNA Polymerase	DNA	●		30°C	10 min. at 65°C

The use of these products in certain applications, some additives or protocols may be covered by patents. The user has to analyse all applicable Limited Use Label Licenses and may need licensing for certain cases.

ALLin™ Isothermal DNA Amplification Kit

bulk quantities available

CAT.#	SIZE	COMPONENTS	COMPONENT COMPOSITION
IDK0101	100 r of 25 µl	1.25 ml - ALLin™ Isothermal Amplification Mix, 2X 0.125 ml - Quantitative Fluorescent Dye, 20X 1 ml - PCR Water	1X amplification mix includes recombinant Bst DNA Polymerase (large fragment), 3 mM $MgSO_4$, 1.6 mM dNTPs, enhancers, stabilizers. Quantitative Fluorescent Dye, 20X is to be used if the reaction is run in qPCR cyclers and real-time detection is performed in FAM channel.
IDK0105	500 r of 25 µl	5 x 1.25 ml - ALLin™ Isothermal Amplification Mix, 2X 5 x 0.125 ml - Quantitative Fluorescent Dye, 20X 5 x 1 ml - PCR Water	

Storage: *In the dark at -20°C.*

APPLICATIONS

- Isothermal DNA amplification at elevated temperature
- Real-time detection of DNA amplification
- LAMP - loop-mediated isothermal amplification
- WGA - whole genome amplification
- RAM - ramification amplification

BENEFITS

- Efficient 20 min DNA amplification between 55-70°C
- ALLin™ format, supplied with water and dye for real-time detection
- Excellent strand displacement activity of the Bst DNA Polymerase
- Robust on complex templates and crude samples
- Low-copy (<5 molecules) target detection

PRODUCT DETAILS

ALLin™ Isothermal DNA Amplification Kit enables rapid detection (~20 minutes) of as little as 5 DNA target molecules - even without a thermal cycler. A 2X master mix with optimized high-performance buffer, dNTPs, a recombinant Bst Polymerase large fragment and PCR water is included. Only templates and primers have to be supplied by the user. The Bst Polymerase has excellent 5' - 3' strand displacement activity and efficient 5'-3' polymerase activity at 55-70°C. It has neither 5' - 3' nor 3' - 5' exonuclease activity and retains only minor reverse transcription activity. Quantitative Fluorescent Dye 20X is included for real-time detection in the FAM channel on any qPCR cycler. Efficient amplification of complex GC rich templates is made possible by the ability to run reactions at higher temperatures.

PERFORMANCE

Technical characteristics of Bst DNA Polymerase large fragment:

- Excellent 5' - 3' strand displacement and polymerase activity
- No 5' - 3' or 3' - 5' (proofreading) exonuclease activity
- Minor reverse transcriptase activity (for RNA, use ALLin™ Isothermal 1Step RNA Amplification Kit (#IRK0101) which includes RTase)
- Optimal amplification temperature is 65°C.
- Working temperature range is 55-70°C.
- Optimal reaction time is 20 minutes, if needed, the reaction can be performed 30 to 60 minutes.
- The enzyme is inactivated in 10 minutes at 80°C.
- Quantitative Fluorescent Dye has an excitation max. of 482 nm and emission max. of 512 nm.

The use of this product in certain applications, some additives or protocols may be covered by patents. The user has to analyse all applicable Limited Use Label Licenses and may need licensing for certain cases.

PROTOCOL

- Follow standard laboratory practices to prevent contamination.
- Include a no-template control and positive controls in parallel.
- Thaw and keep reagents on ice. Mix well before use.
- Perform reaction at 65°C or optimize the reaction temperature between 55-70°C for each template/primers system. Complex templates may require a higher temperature.
- Suggested reaction time is 20-30 minutes. For some low copy number targets 30-60 minutes might be required.
- Quantitative Fluorescent Dye, 20X should only be used when performing the reaction in a real-time cycler. Detection is performed in FAM channel, acquiring data every 15 seconds.
- Design primers with predicted melting temperature of approximately 60°C.
- Prepare 10X primer mix in water or TE Buffer.

✓ Prepare a 25 µl reaction:

ALLin™ Isothermal Amplification Mix, 2X	12.5 µl
Optional: Quantitative Fluorescent Dye, 20X	1.25 µl
10X Primer Mix (variable)	2.5 µl
Template DNA (variable)	1 µl
PCR Water (supplied)	To 25 µl

✓ Mix gently, avoid bubbles.
✓ Place into the thermostat or qPCR instrument to incubate

Amplification: 65°C – 20-30 min

Optional Inactivation: 80°C - 10 min

✓ Store reactions for a short time on ice or for long durations at -20°C.

✓ High yield amplification product may increase the viscosity of the solution, mix well and dilute if required for downstream applications.

IN VITRO RESEARCH USE ONLY

ALLin™ Isothermal 1Step RNA Amplification Kit

bulk quantities available

CAT.#	SIZE	COMPONENTS	COMPONENT COMPOSITION
IRK0101	100 r of 25 µl	1.25 ml - ALLin™ Isothermal Amplification Mix, 2X 0.125 ml - Quantitative Fluorescent Dye, 20X 0.2 ml - RT5 Mix 1 ml - PCR Water	1X amplification mix includes recombinant Bst DNA Polymerase (large fragment), 3 mM MgSO4, 1.6 mM dNTPs, enhancers, stabilizers. RT5 Mix includes thermostable reverse transcriptase (modified MMuLV) and a thermostable RNase inhibitor. Quantitative Fluorescent Dye, 20X is to be used if the reaction is run in qPCR cyclers and real-time detection is performed in FAM channel.
IRK0105	500 r of 25 µl	5 x 1.25 ml - ALLin™ Isothermal Amplification Mix, 2X 5 x 0.125 ml - Quantitative Fluorescent Dye, 20X 5 x 0.2 ml - RT5 Mix 5 x 1 ml - PCR Water	

Storage: *In the dark at -20°C.*

APPLICATIONS

- Molecular diagnostics/SARS-CoV-2 detection
- Isothermal 1 step RNA amplification at elevated temperature
- RT-LAMP – reverse transcription loop-mediated isothermal amplification

BENEFITS

- Efficient 30 minutes 1 Step RNA amplification at 55-70°C.
- ALLin™ format, supplied with water and dye for real-time detection
- Bst DNA polymerase has excellent strand displacement activity
- Robust on complex RNA templates and crude samples
- Low-copy (<5 molecules) target detection

PRODUCT DETAILS

ALLin™ Isothermal 1Step RNA Amplification Kit enables the rapid (~30 minutes) detection of > 5 target RNA molecules without the use of PCR cycler. A blend of a thermostable reverse transcriptase and highly efficient RNase Inhibitor synthesizes cDNA from RNA templates. The cDNA is then amplified by Bst polymerase. A 2X master mix with high-performance buffer, dNTPs, PCR water and a recombinant Bst Polymerase large fragment with excellent 5′ - 3′ strand displacement and efficient 5′-3′ polymerase activity (55-70°C) is included. It has neither 5′ - 3′ nor 3′ - 5′ exonuclease activity and retains only minor reverse transcription activity. Quantitative Fluorescent Dye 20X is included for real-time detection (FAM channel). Efficient amplification of complex GC rich templates is made possible by the ability to run reactions at higher temperatures.

PERFORMANCE

Technical characteristics of Bst DNA Polymerase large fragment:

- Excellent 5′-3′ strand displacement and polymerase activity
- No 5′-3′ or 3′-5′ exonuclease (proofreading) activity
- Minimal reverse transcriptase activity
- Optimal amplification temperature is 65°C

Kit characteristics:

- RT5 Mix includes a thermostable reverse transcriptase (modified MMuLV) that operates at high temperatures and a thermostable RNase inhibitor that protects template RNA from degradation.
- Optimal 1Step RT amplification reaction time is 30 minutes. If required, the reaction can be performed in 30 to 60 minutes.
- Fluorescent Dye has an excitation max. of 482 nm and emission max. of 512 nm.

The use of this product in certain applications, some additives or protocols may be covered by patents. The user has to analyse all applicable Limited Use Label Licenses and may need licensing for certain cases.

PROTOCOL

- Follow standard laboratory practices to prevent contamination.
- Include a no-template control and positive controls in parallel.
- Thaw and keep reagents on ice. Mix well before use.
- Perform reaction at 65°C or optimize between 55-70°C for each template/primers system. Complex templates may require a higher temperature.
- Suggested reaction time is 20-30 minutes. For some low copy number targets 30-60 minutes may be required.
- Quantitative Fluorescent Dye, 20X should only be used when performing the reaction in a real-time cycler. Detection is performed in FAM channel, acquiring data every 15 seconds.
- Design primers with predicted melting temp. of approx. 60°C.
- Prepare 10X primer mix in water or TE Buffer.
- Reactions can be stored for a short time on ice

✓ Prepare a 20 µl reaction:

ALLin™ Isothermal Amplification Mix, 2X	12.5 µl
Optional: Quantitative Fluorescent Dye, 20X	1.25 µl
RT5 Mix (Rtase w RNase Inhibitoor)	2 µl
10X Primer Mix (variable)	2.5 µl
Template RNA (variable)	1 µl
PCR Water (supplied)	To 25 µl volume

✓ Mix gently, avoid bubbles.

✓ Place into the thermostat or qPCR instrument to incubate:

Amplification: 65°C - 30 min

Optional Inactivation: 80°C - 10 min

✓ Store reactions for a short time on ice or for long durations at -20°C.

IN VITRO RESEARCH USE ONLY

phi29 DNA Polymerase

bulk quantities available

CAT.#	SIZE	COMPONENTS	COMPONENT COMPOSITION
IDE0101	1000 u	1000 U - phi29 DNA Polymerase, 10 u/µl 0.5 ml – 10X phi29 Buffer	Enzyme storage buffer includes stabilizers and 50% glycerol. 1X phi29 Buffer contains 10 mM (NH4)2SO4, 10 mM MgCl2, 4 mM DTT.
IDE0105	5000 u	5 x 1000 U– phi29 DNA Polymerase, 10 u/µl 5 x 0.5 ml – 10X phi29 Buffer	

Storage: *In the dark at -20°C.*

APPLICATIONS

- Isothermal DNA amplification for sequencing, cloning
- RCA – rolling cycle amplification
- MDA – multiple displacement amplification
- WGA - whole genome amplification
- Protein primed or RNA primed DNA amplification

BENEFITS

- High yield DNA amplification at constant 30°C temperature
- Robust polymerase with excellent strand displacement activity
- High processivity, synthesis of over 70 kb long DNA strands
- High fidelity, low error rate for sequencing and cloning
- Archetypal buffer formulation for known phi29 applications

PRODUCT DETAILS

Recombinant phi29 DNA Polymerase is an enzyme for use in common isothermal DNA amplification applications that are based on strand displacement and are carried out at moderate temperatures. The enzyme is supplied with an optimized high-performance buffer. The user has to add dNTPs, template and primers. The polymerase has excellent strand displacement activity, efficient 5'-3' polymerase activity at approximately 25-37°C, and allows the synthesis of both small and large quantities of DNA. The enzyme has no 5'-3' exonuclease activity but has strong 3' - 5' exonuclease (proofreading) activity that may degrade primers. Therefore, the use of 3' protected exo-resistant primers has been recommended in the literature. The enzyme can be heat-inactivated, tolerates dUTP, and produces blunt-ended DNA.

PERFORMANCE

Most important technical characteristics of phi29 DNA Polymerase (based on abundantly available scientific literature):

- Excellent strand displacement activity
- 5'-3' polymerase activity on DNA/RNA templates
- Exceptional 3' - 5' (proofreading) exonuclease activity
- No 5' - 3' exonuclease activity
- Optimal reaction temperature is 30°C
- Working temperature range is 25-37°C, depending on application
- Optimal reaction time depends on application
- The enzyme is inactivated in 10 minutes at 65°C.
- Addition of pyrophosphatase may accelerate the reaction
- High fidelity, low error rate for sequencing and cloning

The use of this product in certain applications, some additives or protocols may be covered by patents. The user has to analyse all applicable Limited Use Label Licenses and may need licensing for certain cases.

PROTOCOL

- Follow standard laboratory practices to prevent contamination (keep the bench clean, wear gloves, use sterile tubes and filter pipet tips etc.).
- Include a no-template control and positive controls in parallel.
- Thaw and keep reagents on ice. Mix well before use.
- Perform denaturation/primer annealing by heating the reaction to 95°C and cooling it down. Only then add the phi29 Polymerase to start the synthesis.
- Perform the reaction at 30°C. If required, optimize the reaction temperature between 25-37°C for each template/primer system.
- Suggested reaction time is 60-120 minutes. For some low copy number targets, overnight incubation might be required.
- Since abundant amplification product may increase the viscosity of the solution, mix well and dilute if needed for downstream applications.

IN VITRO RESEARCH USE ONLY

An example for enzyme and buffer amounts to prepare a 20 µl reaction:

10X phi29 Buffer	2 µl
10 mM dNTP Mix (#NUM0201)	1 µl
100 µM Primers	1 µl
Template DNA (variable, depends on application)	1 µl (from 1 ng)
PCR Water (#WAT0110)	to 19 µl

✓ Mix gently, avoid bubbles.
✓ Place into the qPCR instrument to anneal primers:

Denaturation/primer annealing	95°C – 3-5 min
Cool down on ice or in the cycler	4°C – 2-3 min
Add phi29 DNA Polymerase	1 µl (10 units)

✓ For DNA synthesis, mix and incubate at 30°C for 1 hour
✓ Enzyme is inactivated for 10 minutes at 65°C.
✓ Reactionws can be stored for a short time on ice or for long durations at -20°C.

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qPCR and HRM Master Mixes

qPCR Selection: Instrument Compatibility of Probe and Dye-based ORA™ qPCR Mixes

Probe-based qPCR				Green dye-based qPCR	
ORA™ (SEE) qPCR Probe	ORA™ (SEE) qPCR Probe ROX L	ORA™ (SEE) qPCR Probe ROX H	Instruments	ORA™ (SEE) qPCR Green ROX L	ORA™ (SEE) qPCR Green ROX H
page 15-16	*page 17-18*	*page 19-20*		*page 21-22*	*page 23-24*
●	●		**Analytic Jena**: qTOWER, qTOWER 2.x **BioRad**: Opticon®, Opticon®2, Chromo4™, MiniOpticon™, CFX96™, CFX384™ **Cepheid**: SmartCycler® **BJS**: Xxpress® **Illumina / PCRmax**: Eco **Eppendorf**: Mastercycler® ep realplex, Mastercycler® realplex 2S **Hain Lifescience**: FluoroCycler®96 **IT-IS Life Science**: MyGo Pro, MyGo Mini **QIAGEN**: Rotor-Gene®Q, Rotor-Gene® 6000, Rotor-Gene® 3000 **Roche Applied Science**: LightCycler®480, LightCycler®96, LightCycler®Nano **Takara**: Thermal Cycler Dice® **Thermo Fisher Scientific**: Piko Real® **Techne**: PrimeQ, Quantica®	●	
	●		**Agilent**: AriaMX, Mx3000P®, Mx3005P®, Mx4000P® **Fluidigm**: BioMark™ **Thermo Fisher Scientific**: 7500, 7500 FAST, Viia™7, QuantStudio™ 3 / 5 / 6 / 7 / 12K Flex	●	
		●	**Thermo Fisher Scientific**: 7000, 7300, 7700, 7900, 7900HT, 7900HT FAST, StepOne™, StepOnePlus™		●
●			**BioRad**: iCycler®, MyiQ™, iQ™5		

High Resolution Melting Analysis Selection: Instrument Compatibility of ORA™ qPCR HRM Mix *(page 25)*

Agilent:	AriaMX
BioRad:	CFX96™, CFX384™
Eppendorf:	Mastercycler® ep realplex Mastercycler® realplex 2S
Illumina / PCRmax:	Eco
QIAGEN:	Rotor-Gene®Q, Rotor-Gene® 6000
Roche Applied Science:	LightCycler®480, LightCycler®96, LightCycler®Nano
Thermo Fisher Scientific:	StepOne™, StepOnePlus™, 7500 FAST, 7900HT FAST, Viia7™, QuantStudio™ 6 / 7/ 12K Flex

ORA™ qPCR Probe Mix, 2X

bulk quantities available

CAT.#	SIZE	COMPONENTS	COMPONENT COMPOSITION
QPP0101	200 r of 20 µl	2 x 1 ml - ORA™ qPCR Probe Mix, 2X 2 x 1 ml - PCR Water	Hot Start qPCR components: dNTPs at 0.25 mM, optimized buffer, ROX is not included.
QPP0105	1000 r of 20 µl	10 x 1 ml - ORA™ qPCR Probe Mix, 2X 10 x 1 ml - PCR Water	
Storage:	*In the dark at -20°C.*		

APPLICATIONS

- qPCR assays based on specific probes: including TaqMan®, Molecular Beacons, Scorpions™ Probes
- Quantification of gDNA, cDNA, viral DNA, low copy number genes, gene expression analysis

BENEFITS

- Universal - both standard and fast cycling, all probe qPCR assays, GC or AT rich templates
- Excellent for both single-plex & multiplexing
- Rapid extension, early Ct
- Supplied with PCR Water for maximum convenience

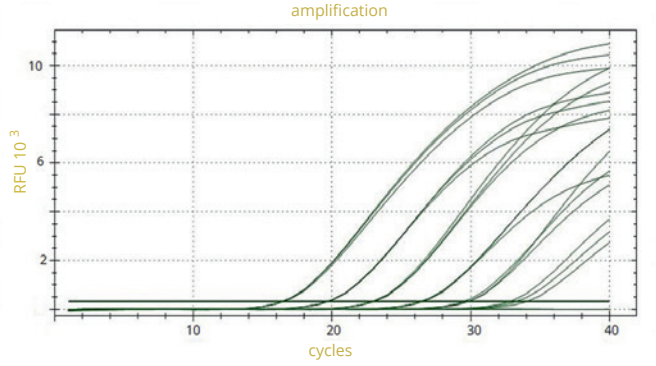
PRODUCT DETAILS

highQu qPCR mastermixes are based on the small molecular inhibitor technology Hot Start PCR allowing to achieve highest sensitivity and specificity under both standard and fast qPCR cycling conditions. They provide excellent results on both AT and GC rich templates, in multiplexing and guaranty rapid extension with early Ct values with minimum or no optimization.

Our mastermixes are supplied with PCR Water to guaranty the best performance. To suit the broad instrument range the ORA™ qPCR Probe Mixes are available in three versions – without ROX, with low or high ROX concentration.
See the selection table on page 14.

PERFORMANCE

ORA™ qPCR Probe Mix provides high sensitivity 100% efficiency qPCR from 10 copies of the target: TaqMan™ probe amplification traces from plasmid dilution series of 1x106 copies to 10 copies of DNA.
95 oC 2 m, 40 x 95 oC 10 s & 60 oC 15 s, Biorad CFX. Human gene ACVR2B.



PROTOCOL

- Use special primer selection programs for good planning.
- Work with amplicons in a range of 80-200, max 400 bp.
- Take typical measures to prevent PCR cross over contamination, keep your bench clean, wear gloves, use sterile tubes and filter pipet tips.
- Run reactions in triplets; include a no-template control and positive control in parallel.
- Thaw and keep reagents on ice. Mix well before use.
- Do not perform annealing/extension for more than 30 seconds and do not use lower than 60 °C temperature for this step.

IN VITRO RESEARCH USE ONLY

✓ Prepare a 20 µl reaction:

Reverse Primer	100-400 nM final c.
Forward Primer	100-400 nM final c.
Specific Probe	200 nM final c. (0.4 µl of 10 µM)
cDNA Template or gDNA Template	<100 ng or 1 µg
PCR Water	to 10 µl
ORA™ qPCR Mix, 2X	10 µl

✓ Mix gently, avoid bubbles.
✓ Place into the instrument set like:

Initial denaturation	1 cycle: 95°C - 2 min for cDNA, or 1 cycle: 95°C - 3 min for gDNA
Denaturation	40 cycles: 95°C - 5 sec
Annealing/extension	40 cycles: 60 - 65°C – 20 - 30 sec

✓ Follow instrument instructions for melting curve analysis.

For optional use, the ROX passive reference dye is premixed within the ROX L and ROX H qPCR Mixes. If the purchaser has an instrument capable of optional ROX detection and wishes to perform the optional normalization of the signal, then the user must select the option in the software.
Notice to Purchaser: With purchasing of this product, no rights are conveyed with respect to U.S. Patent: 5,928,907 and corresponding patents outside the US.

ORA™ SEE qPCR Probe Mix, 2X

bulk quantities available

CAT.#	SIZE	COMPONENTS	COMPONENT COMPOSITION
QPP0401	200 r of 20 µl	2 x 1 ml - ORA™ SEE qPCR Probe Mix, 2X 2 x 1 ml - PCR Water	Mix includes an inert blue dye for better visibility, Hot Start qPCR components: dNTPs at 0.25 mM, optimized buffer; ROX is not included.
QPP0405	1000 r of 20 µl	10 x 1 ml - ORA™ SEE qPCR Probe Mix, 2X 10 x 1 ml - PCR Water	
Storage:	*In the dark at -20°C.*		

APPLICATIONS

- qPCR assays based on specific probes: including TaqMan®, Molecular Beacons, Scorpions™ Probes
- Quantification of gDNA, cDNA, viral DNA, low copy number genes, gene expression analysis

BENEFITS

- Universal - both standard and fast cycling, all probe qPCR assays, GC or AT rich templates
- Excellent for both single-plex & multiplexing
- Rapid extension, early Ct
- Inert blue dye for a better sample visibility and tracking

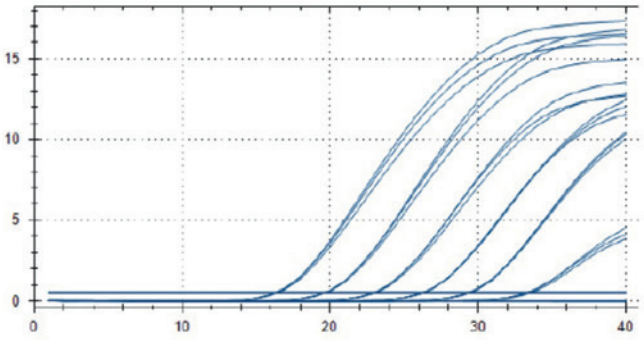
PRODUCT DETAILS

highQu qPCR mastermixes are based on the small molecular inhibitor technology Hot Start PCR allowing to achieve highest sensitivity and specificity under both standard and fast qPCR cycling conditions. They provide excellent results on both AT and GC rich templates, in multiplexing and guaranty rapid extension with early Ct values with minimum or no optimization. ORA™ SEE qPCR mixes provide an additional advantage of a simplified tracking of the process, as they are colored with an inert blue dye to make samples much better visible during pipetting and handling.

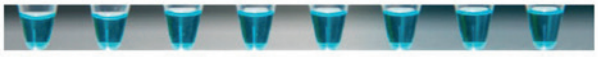
Our mastermixes are supplied with PCR Water to guaranty the best performance. To suit the broad instrument range the ORA™ qPCR Probe Mixes are available in three versions – without ROX, with low or high ROX concentration.

See the selection table on page 14.

PERFORMANCE



Visible blue samples, 100% efficiency, 10 copies detection sensitivity achieved with ORA™ SEE qPCR Probe Mixes. *TaqMan® probe amplification with ORA™ SEE qPCR Probe Mix from plasmid dilution series (1 x 10^6 to 10 copies).*



PROTOCOL

- Use special primer selection programs for good planning.
- Work with amplicons in a range of 80-200, max 400 bp.
- Take typical measures to prevent PCR cross over contamination, keep your bench clean, wear gloves, use sterile tubes and filter pipet tips.
- Run reactions in triplets; include a no-template control and positive control in parallel.
- Thaw and keep reagents on ice. Mix well before use.
- Do not perform annealing/extension for more than 30 seconds and do not use lower than 60 °C temperature for this step.

IN VITRO RESEARCH USE ONLY

✓ Prepare a 20 µl reaction:

Reverse Primer	100-400 nM final c.
Forward Primer	100-400 nM final c.
Specific Probe	200 nM final c. (0.4 µl of 10 µM)
cDNA Template or gDNA Template	<100 ng or 1 µg
PCR Water	to 10 µl
ORA™ SEE qPCR Mix, 2X	10 µl

✓ Mix gently, avoid bubbles.
✓ Place into the instrument set like:

Initial denaturation	1 cycle: 95°C - 2 min for cDNA, or 1 cycle: 95°C - 3 min for gDNA
Denaturation	40 cycles: 95°C - 5 sec
Annealing/extension	40 cycles: 60 - 65°C – 20 - 30 sec

✓ Follow instrument instructions for melting curve analysis.

For optional use, the ROX passive reference dye is premixed within the ROX L and ROX H qPCR Mixes. If the purchaser has an instrument capable of optional ROX detection and wishes to perform the optional normalization of the signal, then the user must select the option in the software.

ORA™ qPCR Probe ROX L Mix, 2X

bulk quantities available

CAT.#	SIZE	COMPONENTS	COMPONENT COMPOSITION
QPP0201	200 r of 20 µl	2 x 1 ml - ORA™ qPCR Probe ROX L Mix, 2X 2 x 1 ml - PCR Water	Hot Start qPCR components: dNTPs at 0.25 mM, optimized buffer, low ROX concentration.
QPP0205	1000 r of 20 µl	10 x 1 ml - ORA™ qPCR Probe ROX L Mix, 2X 10 x 1 ml - PCR Water	
Storage:	*In the dark at -20°C.*		

APPLICATIONS

- qPCR assays based on specific probes: including TaqMan®, Molecular Beacons, Scorpions™ Probes
- Quantification of gDNA, cDNA, viral DNA, low copy number genes, gene expression analysis

BENEFITS

- Universal - both standard and fast cycling, all probe qPCR assays, GC or AT rich templates
- Excellent for both single-plex & multiplexing
- Rapid extension, early Ct
- Supplied with PCR Water for maximum convenience

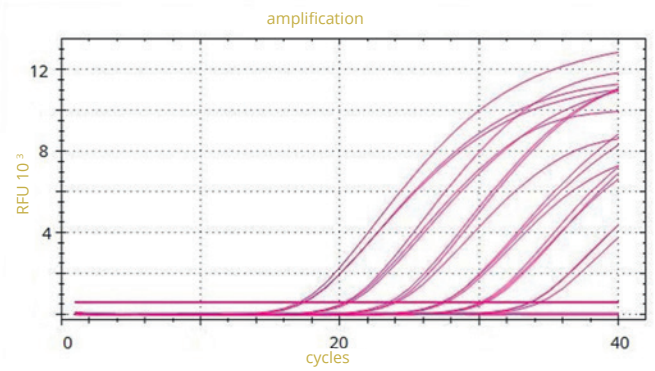
PRODUCT DETAILS

highQu qPCR mastermixes are based on the small molecular inhibitor technology Hot Start PCR allowing to achieve highest sensitivity and specificity under both standard and fast qPCR cycling conditions. They provide excellent results on both AT and GC rich templates, in multiplexing and guaranty rapid extension with early Ct values with minimum or no optimization.

Our mastermixes are supplied with PCR Water to guaranty the best performance. To suit the broad instrument range the ORA™ qPCR Probe Mixes are available in three versions – without ROX, with low or high ROX concentration.
See the selection table on page 14.

PERFORMANCE

ORA™ qPCR Probe Mix provides high sensitivity 100% efficiency qPCR from 10 copies of the target: TaqMan® probe amplification traces from plasmid dilution series of 1x10⁶ copies to 10 copies of DNA.
95 °C 2 m, 40 x 95 °C 10 s & 60 °C 15 s, Biorad CFX. Human gene LIMK1.



PROTOCOL

- Use special primer selection programs for good planning.
- Work with amplicons in a range of 80-200, max 400 bp.
- Take typical measures to prevent PCR cross over contamination, keep your bench clean, wear gloves, use sterile tubes and filter pipet tips.
- Run reactions in triplets; include a no-template control and positive control in parallel.
- Thaw and keep reagents on ice. Mix well before use.
- Do not perform annealing/extension for more than 30 seconds and do not use lower than 60 °C temperature for this step.

IN VITRO RESEARCH USE ONLY

✓ Prepare a 20 µl reaction:

Reverse Primer	100-400 nM final c.
Forward Primer	100-400 nM final c.
Specific Probe	200 nM final c. (0.4 µl of 10 µM)
cDNA Template or gDNA Template	<100 ng or 1 µg
PCR Water	to 10 µl
ORA™ qPCR Mix, 2X	10 µl

✓ Mix gently, avoid bubbles.
✓ Place into the instrument set like:

Initial denaturation	1 cycle: 95°C - 2 min for cDNA, or 1 cycle: 95°C - 3 min for gDNA
Denaturation	40 cycles: 95°C - 5 sec
Annealing/extension	40 cycles: 60 - 65°C – 20 - 30 sec

✓ Follow instrument instructions for melting curve analysis.

For optional use, the ROX passive reference dye is premixed within the ROX L and ROX H qPCR Mixes. If the purchaser has an instrument capable of optional ROX detection and wishes to perform the optional normalization of the signal, then the user must select the option in the software.
Notice to Purchaser: With purchasing of this product, no rights are conveyed with respect to U.S. Patent: 5,928,907 and corresponding patents outside the US.

ORA™ SEE qPCR Probe ROX L Mix, 2X

bulk quantities available

CAT.#	SIZE	COMPONENTS	COMPONENT COMPOSITION
QPP0501	200 r of 20 µl	2 x 1 ml - ORA™ SEE qPCR Probe ROX L Mix, 2X 2 x 1 ml - PCR Water	Mix includes an inert blue dye for better visibility, Hot Start qPCR components: dNTPs at 0.25 mM, optimized buffer; low ROX concentration.
QPP0505	1000 r of 20 µl	10 x 1 ml - ORA™ SEE qPCR Probe ROX L Mix, 2X 10 x 1 ml - PCR Water	
Storage:	*In the dark at -20°C.*		

APPLICATIONS

- qPCR on instruments calibrated with low ROX conc.
- qPCR assays based on specific probes: including TaqMan®, Molecular Beacons, Scorpions™ Probes
- Quantification of gDNA, cDNA, viral DNA, low copy number genes, gene expression analysis

BENEFITS

- Universal - both standard and fast cycling, all probe qPCR assays, GC or AT rich templates
- Excellent for both single-plex & multiplexing
- Rapid extension, early Ct
- Inert blue dye for a better sample visibility and tracking

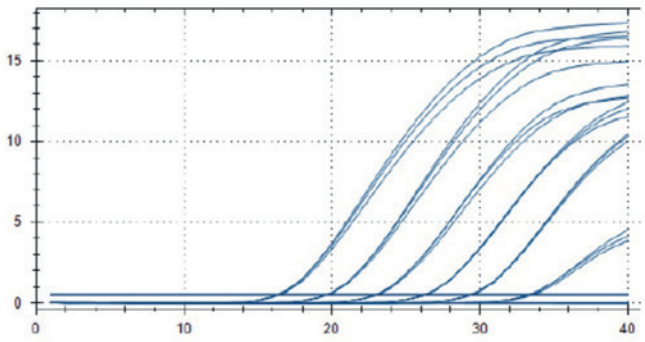
PRODUCT DETAILS

highQu qPCR mastermixes are based on the small molecular inhibitor technology Hot Start PCR allowing to achieve highest sensitivity and specificity under both standard and fast qPCR cycling conditions. They provide excellent results on both AT and GC rich templates, in multiplexing and guaranty rapid extension with early Ct values with minimum or no optimization. ORA™ SEE qPCR mixes provide an additional advantage of a simplified tracking of the process, as they are colored with an inert blue dye to make samples much better visible during pipetting and handling.

Our mastermixes are supplied with PCR Water to guaranty the best performance. To suit the broad instrument range the ORA™ qPCR Probe Mixes are available in three versions – without ROX, with low or high ROX concentration.

See the selection table on page 14.

PERFORMANCE



Visible blue samples, 100% efficiency, 10 copies detection sensitivity achieved with ORA™ SEE qPCR Probe Mixes. *TaqMan® probe amplification with ORA™ SEE qPCR Probe Mix from plasmid dilution series (1 x 10^6 to 10 copies).*

PROTOCOL

- Use special primer selection programs for good planning.
- Work with amplicons in a range of 80-200, max 400 bp.
- Take typical measures to prevent PCR cross over contmination, keep your bench clean, wear gloves, use sterile tubes and filter pipet tips.
- Run reactions in triplets; include a no-template control and positive control in parallel.
- Thaw and keep reagents on ice. Mix well before use.
- Do not perform annealing/extension for more than 30 seconds and do not use lower than 60 °C temperature for this step.

IN VITRO RESEARCH USE ONLY

✓ Prepare a 20 µl reaction:

Reverse Primer	100-400 nM final c.
Forward Primer	100-400 nM final c.
Specific Probe	200 nM final c. (0.4 µl of 10 µM)
cDNA Template or gDNA Template	<100 ng or 1 µg
PCR Water	to 10 µl
ORA™ SEE qPCR Mix, 2X	10 µl

✓ Mix gently, avoid bubbles.
✓ Place into the instrument set like:

Initial denaturation	1 cycle: 95°C - 2 min for cDNA, or 1 cycle: 95°C - 3 min for gDNA
Denaturation	40 cycles: 95°C - 5 sec
Annealing/extension	40 cycles: 60 - 65°C – 20 - 30 sec

✓ Follow instrument instructions for melting curve analysis.

For optional use, the ROX passive reference dye is premixed within the ROX L and ROX H qPCR Mixes. If the purchaser has an instrument capable of optional ROX detection and wishes to perform the optional normalization of the signal, then the user must select the option in the software.

ORA™ qPCR Probe ROX H Mix, 2X

bulk quantities available

CAT.#	SIZE	COMPONENTS	COMPONENT COMPOSITION
QPP0301	200 r of 20 µl	2 x 1 ml - ORA™ qPCR Probe ROX H Mix, 2X 2 x 1 ml - PCR Water	Hot Start qPCR components: dNTPs at 0.25 mM, optimized buffer, high ROX concentration.
QPP0305	200 r of 20 µl	10 x 1 ml - ORA™ qPCR Probe ROX H Mix, 2X 10 x 1 ml - PCR Water	
Storage:	*In the dark at -20°C.*		

APPLICATIONS

- qPCR assays based on specific probes: including TaqMan®, Molecular Beacons, Scorpions™ Probes
- Quantification of gDNA, cDNA, viral DNA, low copy number genes, gene expression analysis

BENEFITS

- Universal - both standard and fast cycling, all probe qPCR assays, GC or AT rich templates
- Excellent for both single-plex & multiplexing
- Rapid extension, early Ct
- Supplied with PCR Water for maximum convenience

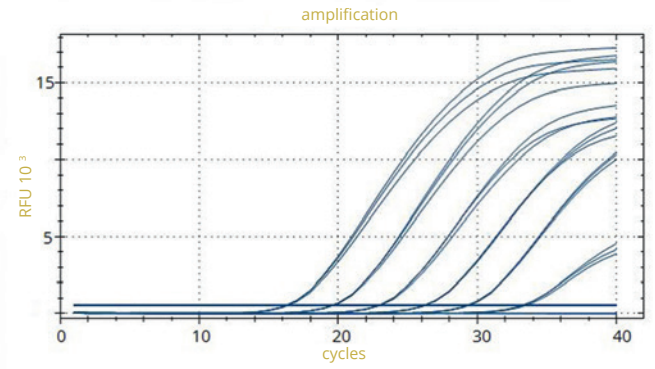
PRODUCT DETAILS

highQu qPCR mastermixes are based on the small molecular inhibitor technology Hot Start PCR allowing to achieve highest sensitivity and specificity under both standard and fast qPCR cycling conditions. They provide excellent results on both AT and GC rich templates, in multiplexing and guaranty rapid extension with early Ct values with minimum or no optimization.

Our mastermixes are supplied with PCR Water to guaranty the best performance. To suit the broad instrument range the ORA™ qPCR Probe Mixes are available in three versions – without ROX, with low or high ROX concentration.
See the selection table on page 14.

PERFORMANCE

ORA™ qPCR Probe Mix provides high sensitivity 100% efficiency qPCR from 10 copies of the target: TaqMan® probe amplification traces from plasmid dilution series of 1x10⁶ copies to 10 copies of DNA.
95 °C 2 m, 40 x 95 °C 10 s & 60 °C 15 s, Biorad CFX. Human gene ACVR1B.



PROTOCOL

- Use special primer selection programs for good planning.
- Work with amplicons in a range of 80-200, max 400 bp.
- Take typical measures to prevent PCR cross over contamination, keep your bench clean, wear gloves, use sterile tubes and filter pipet tips.
- Run reactions in triplets; include a no-template control and positive control in parallel.
- Thaw and keep reagents on ice. Mix well before use.
- Do not perform annealing/extension for more than 30 seconds and do not use lower than 60 °C temperature for this step.

✓ Prepare a 20 µl reaction:

Reverse Primer	100-400 nM final c.
Forward Primer	100-400 nM final c.
Specific Probe	200 nM final c. (0.4 µl of 10 µM)
cDNA Template or gDNA Template	<100 ng or 1 µg
PCR Water	to 10 µl
ORA™ qPCR Mix, 2X	10 µl

✓ Mix gently, avoid bubbles.
✓ Place into the instrument set like:

Initial denaturation	1 cycle: 95°C - 2 min for cDNA, or 1 cycle: 95°C - 3 min for gDNA
Denaturation	40 cycles: 95°C - 5 sec
Annealing/extension	40 cycles: 60 - 65°C – 20 - 30 sec

✓ Follow instrument instructions for melting curve analysis.

IN VITRO RESEARCH USE ONLY

For optional use, the ROX passive reference dye is premixed within the ROX L and ROX H qPCR Mixes. If the purchaser has an instrument capable of optional ROX detection and wishes to perform the optional normalization of the signal, then the user must select the option in the software.
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ORA™ SEE qPCR Probe ROX H Mix, 2X

bulk quantities available

CAT.#	SIZE	COMPONENTS	COMPONENT COMPOSITION
QPP0601	200 r of 20 µl	2 x 1 ml - ORA™ SEE qPCR Probe ROX H Mix, 2X 2 x 1 ml - PCR Water	Mix includes an inert blue dye for better visibility, Hot Start qPCR components: dNTPs at 0.25 mM, optimized buffer; high ROX concentration.
QPP0605	200 r of 20 µl	10 x 1 ml - ORA™ SEE qPCR Probe ROX H Mix, 2X 10 x 1 ml - PCR Water	
Storage:	*In the dark at -20°C.*		

APPLICATIONS

- qPCR on instruments calibrated with high ROX conc.
- qPCR assays based on specific probes: including TaqMan®, Molecular Beacons, Scorpions™ Probes
- Quantification of gDNA, cDNA, viral DNA, low copy number genes, gene expression analysis

BENEFITS

- Universal - both standard and fast cycling, all probe qPCR assays, GC or AT rich templates
- Excellent for both single-plex & multiplexing
- Rapid extension, early Ct
- Inert blue dye for a better sample visibility and tracking

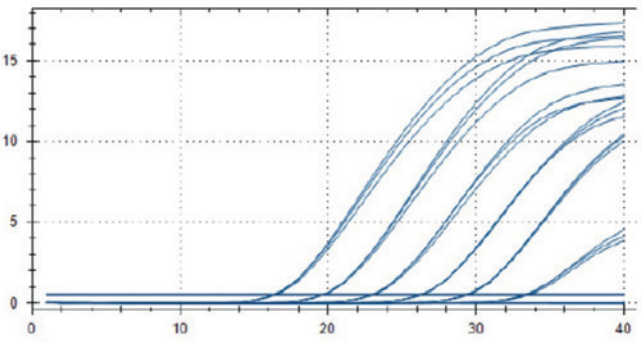
PRODUCT DETAILS

highQu qPCR mastermixes are based on the small molecular inhibitor technology Hot Start PCR allowing to achieve highest sensitivity and specificity under both standard and fast qPCR cycling conditions. They provide excellent results on both AT and GC rich templates, in multiplexing and guaranty rapid extension with early Ct values with minimum or no optimization. ORA™ SEE qPCR mixes provide an additional advantage of a simplified tracking of the process, as they are colored with an inert blue dye to make samples much better visible during pipetting and handling.

Our mastermixes are supplied with PCR Water to guaranty the best performance. To suit the broad instrument range the ORA™ qPCR Probe Mixes are available in three versions – without ROX, with low or high ROX concentration.

See the selection table on page 14.

PERFORMANCE



Visible blue samples, 100% efficiency, 10 copies detection sensitivity achieved with ORA™ SEE qPCR Probe Mixes. *TaqMan® probe amplification with ORA™ SEE qPCR Probe Mix from plasmid dilution series (1 x 10^6 to 10 copies).*

PROTOCOL

- Use special primer selection programs for good planning.
- Work with amplicons in a range of 80-200, max 400 bp.
- Take typical measures to prevent PCR cross over contamination, keep your bench clean, wear gloves, use sterile tubes and filter pipet tips.
- Run reactions in triplets; include a no-template control and positive control in parallel.
- Thaw and keep reagents on ice. Mix well before use.
- Do not perform annealing/extension for more than 30 seconds and do not use lower than 60 °C temperature for this step.

IN VITRO RESEARCH USE ONLY

✓ Prepare a 20 µl reaction:

Reverse Primer	100-400 nM final c.
Forward Primer	100-400 nM final c.
Specific Probe	200 nM final c. (0.4 µl of 10 µM)
cDNA Template or gDNA Template	<100 ng or 1 µg
PCR Water	to 10 µl
ORA™ qPCR Mix, 2X	10 µl

✓ Mix gently, avoid bubbles.
✓ Place into the instrument set like:

Initial denaturation	1 cycle: 95°C - 2 min for cDNA, or 1 cycle: 95°C - 3 min for gDNA
Denaturation	40 cycles: 95°C - 5 sec
Annealing/extension	40 cycles: 60 - 65°C – 20 - 30 sec

✓ Follow instrument instructions for melting curve analysis.

For optional use, the ROX passive reference dye is premixed within the ROX L and ROX H qPCR Mixes. If the purchaser has an instrument capable of optional ROX detection and wishes to perform the optional normalization of the signal, then the user must select the option in the software.

ORA™ qPCR Green ROX L Mix, 2X

bulk quantities available

CAT.#	SIZE	COMPONENTS	COMPONENT COMPOSITION
QPD0101	200 r of 20 µl	2 x 1 ml - ORA™ qPCR Green ROX L Mix, 2X 2 x 1 ml - PCR Water	Hot Start qPCR components: dNTPs at 0.25 mM, optimized buffer, low ROX concentration.
QPD0105	1000 r of 20 µl	10 x 1 ml - ORA™ qPCR Green ROX L Mix, 2X 10 x 1 ml - PCR Water	
Storage:	*In the dark at -20°C.*		

APPLICATIONS

- qPCR from gDNA, cDNA, viral DNA, low copy number genes
- Relative gene expression analysis, absolute quantification
- qPCR on instruments calibrated with low ROX conc.
- qPCR assays based on fluorescence of intercalating dye

BENEFITS

- Universal - standard and fast cycling, GC or AT rich templates
- Highest sensitivity, rapid extension, early Ct values
- Supplied with PCR Water for maximum convenience

PRODUCT DETAILS

highQu qPCR mastermixes are based on the small molecular inhibitor technology Hot Start PCR allowing to achieve highest sensitivity and specificity under both standard and fast qPCR cycling conditions. They provide excellent results on both AT and GC rich templates and guaranty rapid extension with early Ct values with minimum or no optimization.

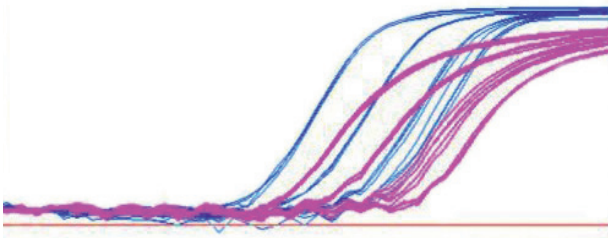
Our mastermixes are supplied with PCR Water to guaranty the best performance. To suit the broad instrument range the ORA™ qPCR Green Mixes are available in different versions – with low or high ROX concentration.

See the selection table on page 14.

PERFORMANCE

ORA™ qPCR Green Mix (blue curves) provides in many cases earlier Ct values compared to competitor mastermixes.

Conditions: 95 °C 2 m, 40 x 95 °C 10 s & 60 °C 15 s, Roche LightCycler® 480. Amplification of mouse ACTG1 from cDNA dilution series.



PROTOCOL

- Use special primer selection programs for good planning.
- Work with amplicons in a range of 80-200, max 400 bp.
- Take typical measures to prevent PCR cross over contmination, keep your bench clean, wear gloves, use sterile tubes and filter pipet tips.
- Run reactions in triplets; include a no-template control and positive control in parallel.
- Thaw and keep reagents on ice. Mix well before use.
- Do not perform annealing/extension for more than 30 seconds and do not use lower than 60 °C temperature for this step.

IN VITRO RESEARCH USE ONLY

✓ Prepare a 20 µl reaction:

Reverse Primer	100-400 nM final c.
Forward Primer	100-400 nM final c.
cDNA Template or gDNA Template	<100 ng or 1 µg
PCR Water	to 10 µl
ORA™ qPCR Mix, 2X	10 µl

✓ Mix gently, avoid bubbles.

✓ Place into the instrument (SYBR® Green or FAM channel) set like:

Initial denaturation	1 cycle: 95°C - 2 min for cDNA, or 1 cycle: 95°C - 3 min for gDNA
Denaturation	40 cycles: 95°C - 5 sec
Annealing/extension	40 cycles: 60 - 65°C – 20 - 30 sec

✓ Follow instrument instructions for melting curve analysis.

For optional use, the ROX passive reference dye is premixed within the ROX L and ROX H qPCR Mixes. If the purchaser has an instrument capable of optional ROX detection and wishes to perform the optional normalization of the signal, then the user must select the option in the software.

ORA™ SEE qPCR Green ROX L Mix, 2X

bulk quantities available

CAT.#	SIZE	COMPONENTS	COMPONENT COMPOSITION
QPD0501	200 r of 20 µl	2 x 1 ml - ORA™ SEE qPCR Green ROX L Mix, 2X 2 x 1 ml - PCR Water	Mix includes an inert blue dye for better visibility, Hot Start qPCR components: dNTPs at 0.25 mM, optimized buffer, low ROX concentration.
QPD0505	1000 r of 20 µl	10 x 1 ml - ORA™ SEE qPCR Green ROX L Mix, 2X 10 x 1 ml - PCR Water	
Storage:	*In the dark at -20°C.*		

APPLICATIONS

- qPCR from gDNA, cDNA, viral DNA, low copy number genes
- Relative gene expression analysis, absolute quantification
- qPCR on instruments calibrated with low ROX conc.
- qPCR assays based on fluorescence of intercalating dye

BENEFITS

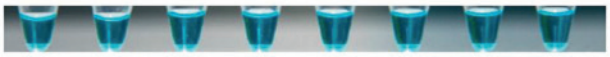
- Universal - both standard and fast cycling, GC or AT rich templates
- Highest sensitivity, rapid extension, early Ct
- Supplied with PCR Water for maximum convenience
- Inert blue dye for a better sample visibility and tracking

PRODUCT DETAILS

highQu qPCR mastermixes are based on the small molecular inhibitor technology Hot Start PCR allowing to achieve highest sensitivity and specificity under both standard and fast qPCR cycling conditions. They provide excellent results on both AT and GC rich templates and guaranty rapid extension with early Ct values with minimum or no optimization.
ORA™ SEE qPCR mixes provide an additional advantage of a simplified tracking of the process, as they are colored with an inert blue dye to make samples much better visible during pipetting and handling.
Our mastermixes are supplied with PCR Water to guaranty the best performance. To suit the broad instrument range the ORA™ qPCR Green Mixes are available in different versions –with low or high ROX concentration.
See the selection table on page 14.

PERFORMANCE

Visible blue samples, earlier Ct values, superb sensitivity achieved with ORA™ SEE qPCR Green Mixes. *Amplification & melt traces of mouse actin gamma-1 housekeeping gene from a cDNA dilution series; ORA™ SEE qPCR Green Mix (purple) and Competitor Mix (black).*



PROTOCOL

- Use special primer selection programs for good planning.
- Work with amplicons in a range of 80-200, max 400 bp.
- Take typical measures to prevent PCR cross over contamination, keep your bench clean, wear gloves, use sterile tubes and filter pipet tips.
- Run reactions in triplets; include a no-template control and positive control in parallel.
- Thaw and keep reagents on ice. Mix well before use.
- Do not perform annealing/extension for more than 30 seconds and do not use lower than 60 °C temperature for this step.

IN VITRO RESEARCH USE ONLY

✓ Prepare a 20 µl reaction:

Reverse Primer	100-400 nM final c.
Forward Primer	100-400 nM final c.
cDNA Template or gDNA Template	<100 ng or 1 µg
PCR Water	to 10 µl
ORA™ SEE qPCR Mix, 2X	10 µl

✓ Mix gently, avoid bubbles.
✓ Place into the instrument (SYBR® Green or FAM channel) set like:

Initial denaturation	1 cycle: 95°C - 2 min for cDNA, or 1 cycle: 95°C - 3 min for gDNA
Denaturation	40 cycles: 95°C - 5 sec
Annealing/extension	40 cycles: 60 - 65°C – 20 - 30 sec

✓ Follow instrument instructions for melting curve analysis.

For optional use, the ROX passive reference dye is premixed within the ROX L and ROX H qPCR Mixes. If the purchaser has an instrument capable of optional ROX detection and wishes to perform the optional normalization of the signal, then the user must select the option in the software.
Notice to Purchaser: With purchasing of this product, no rights are conveyed with respect to U.S. Patent: 5,928,907 and corresponding patents outside the US.

ORA™ qPCR Green ROX H Mix, 2X

bulk quantities available

CAT.#	SIZE	COMPONENTS	COMPONENT COMPOSITION
QPD0201	200 r of 20 µl	2 x 1 ml - ORA™ qPCR Green ROX H Mix, 2X 2 x 1 ml - PCR Water	Hot Start qPCR components: dNTPs at 0.25 mM, optimized buffer, high ROX concentration.
QPD0205	1000 r of 20 µl	10 x 1 ml - ORA™ qPCR Green ROX H Mix, 2X 10 x 1 ml - PCR Water	
Storage:	*In the dark at -20°C.*		

APPLICATIONS

- qPCR from gDNA, cDNA, viral DNA, low copy number genes
- Relative gene expression analysis, absolute quantification
- qPCR on instruments calibrated with high ROX conc.
- qPCR assays based on fluorescence of intercalating dye

BENEFITS

- Universal - standard and fast cycling, GC or AT rich templates
- Highest sensitivity, rapid extension, early Ct values
- Supplied with PCR Water for maximum convenience

PRODUCT DETAILS

highQu qPCR mastermixes are based on the small molecular inhibitor technology Hot Start PCR allowing to achieve highest sensitivity and specificity under both standard and fast qPCR cycling conditions. They provide excellent results on both AT and GC rich templates and guaranty rapid extension with early Ct values with minimum or no optimization.

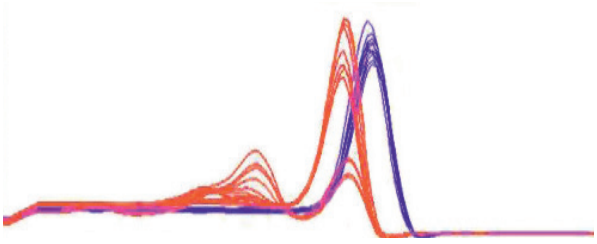
Our mastermixes are supplied with PCR Water to guaranty the best performance. To suit the broad instrument range the ORA™ qPCR Green Mixes are available in different versions – with low or high ROX concentration.

See the selection table on page 14.

PERFORMANCE

ORA™ qPCR Green Mix (blue curves) provides in many cases higher sensitivity compared to competitor mastermixes.

Conditions: 95 °C 2 m, 40 x 95 °C 10 s & 60 °C 15 s, Roche LightCycler® 480. Amplification of mouse ACTG1 from cDNA dilution series.



PROTOCOL

- Use special primer selection programs for good planning.
- Work with amplicons in a range of 80-200, max 400 bp.
- Take typical measures to prevent PCR cross over contamination, keep your bench clean, wear gloves, use sterile tubes and filter pipet tips.
- Run reactions in triplets; include a no-template control and positive control in parallel.
- Thaw and keep reagents on ice. Mix well before use.
- Do not perform annealing/extension for more than 30 seconds and do not use lower than 60 °C temperature for this step.

IN VITRO RESEARCH USE ONLY

✓ Prepare a 20 µl reaction:

Reverse Primer	100-400 nM final c.
Forward Primer	100-400 nM final c.
cDNA Template or gDNA Template	<100 ng or 1 µg
PCR Water	to 10 µl
ORA™ qPCR Mix, 2X	10 µl

✓ Mix gently, avoid bubbles.

✓ Place into the instrument (SYBR® Green or FAM channel) set like:

Initial denaturation	1 cycle: 95°C - 2 min for cDNA, or 1 cycle: 95°C - 3 min for gDNA
Denaturation	40 cycles: 95°C - 5 sec
Annealing/extension	40 cycles: 60 - 65°C – 20 - 30 sec

✓ Follow instrument instructions for melting curve analysis.

For optional use, the ROX passive reference dye is premixed within the ROX L and ROX H qPCR Mixes. If the purchaser has an instrument capable of optional ROX detection and wishes to perform the optional normalization of the signal, then the user must select the option in the software.

ORA™ SEE qPCR Green ROX H Mix, 2X

bulk quantities available

CAT.#	SIZE	COMPONENTS	COMPONENT COMPOSITION
QPD0401	200 r of 20 µl	2 x 1 ml - ORA™ SEE qPCR Green ROX H Mix, 2X 2 x 1 ml - PCR Water	Mix includes an inert blue dye for better visibility, Hot Start qPCR components: dNTPs at 0.25 mM, optimized buffer, high ROX concentration.
QPD0405	1000 r of 20 µl	10 x 1 ml - ORA™ SEE qPCR Green ROX H Mix, 2X 10 x 1 ml - PCR Water	

Storage: *In the dark at -20°C.*

APPLICATIONS

- qPCR from gDNA, cDNA, viral DNA, low copy number genes
- Relative gene expression analysis, absolute quantification
- qPCR on instruments calibrated with high ROX conc.
- qPCR assays based on fluorescence of intercalating dye

BENEFITS

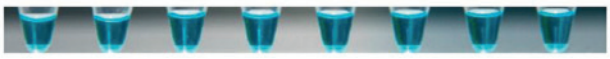
- Universal - both standard and fast cycling, GC or AT rich templates
- Highest sensitivity, rapid extension, early Ct
- Supplied with PCR Water for maximum convenience
- Inert blue dye for a better sample visibility and tracking

PRODUCT DETAILS

highQu qPCR mastermixes are based on the small molecular inhibitor technology Hot Start PCR allowing to achieve highest sensitivity and specificity under both standard and fast qPCR cycling conditions. They provide excellent results on both AT and GC rich templates and guaranty rapid extension with early Ct values with minimum or no optimization.
ORA™ SEE qPCR mixes provide an additional advantage of a simplified tracking of the process, as they are colored with an inert blue dye to make samples much better visible during pipetting and handling.
Our mastermixes are supplied with PCR Water to guaranty the best performance. To suit the broad instrument range the ORA™ qPCR Green Mixes are available in different versions –with low or high ROX concentration.
See the selection table on page 14.

PERFORMANCE

Visible blue samples, earlier Ct values, superb sensitivity achieved with ORA™ SEE qPCR Green Mixes. *Amplification & melt traces of mouse actin gamma-1 housekeeping gene from a cDNA dilution series; ORA™ SEE qPCR Green Mix (purple) and Competitor Mix (black).*



PROTOCOL

- Use special primer selection programs for good planning.
- Work with amplicons in a range of 80-200, max 400 bp.
- Take typical measures to prevent PCR cross over contamination, keep your bench clean, wear gloves, use sterile tubes and filter pipet tips.
- Run reactions in triplets; include a no-template control and positive control in parallel.
- Thaw and keep reagents on ice. Mix well before use.
- Do not perform annealing/extension for more than 30 seconds and do not use lower than 60 °C temperature for this step.

IN VITRO RESEARCH USE ONLY

✓ Prepare a 20 µl reaction:

Reverse Primer	100-400 nM final c.
Forward Primer	100-400 nM final c.
cDNA Template or gDNA Template	<100 ng or 1 µg
PCR Water	to 10 µl
ORA™ SEE qPCR Mix, 2X	10 µl

✓ Mix gently, avoid bubbles.
✓ Place into the instrument (SYBR® Green or FAM channel) set like:

Initial denaturation	1 cycle: 95°C - 2 min for cDNA, or 1 cycle: 95°C - 3 min for gDNA
Denaturation	40 cycles: 95°C - 5 sec
Annealing/extension	40 cycles: 60 - 65°C – 20 - 30 sec

✓ Follow instrument instructions for melting curve analysis.

For optional use, the ROX passive reference dye is premixed within the ROX L and ROX H qPCR Mixes. If the purchaser has an instrument capable of optional ROX detection and wishes to perform the optional normalization of the signal, then the user must select the option in the software.
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ORA™ qPCR HRM Mix, 2X

bulk quantities available

CAT.#	SIZE	COMPONENTS	COMPONENT COMPOSITION
QPD0301	200 r of 20 µl	2 x 1 ml - ORA™ qPCR HRM Mix, 2X 2 x 1 ml - PCR Water	Hot Start qPCR components: dNTPs at 0.25 mM, optimized buffer, proprietary saturating intercalating dye.
QPD0305	1000 r of 20 µl	10 x 1 ml - ORA™ qPCR HRM Mix, 2X 10 x 1 ml - PCR Water	
Storage:	*In the dark at -20°C.*		

APPLICATIONS

High Resolution Melting analysis (HRM):

- Detection of sequence variations
- SNP genotyping
- Methylation analysis
- Mutation scanning

BENEFITS

- Time and costs saving analysis of sequence variations
- Universal - standard or fast cycling, GC or AT rich templates
- Highest sensitivity, no optimization required
- Supplied with PCR Water for maximum convenience

PPRODUCT DETAILS

High Resolution Melting analysis (HRM) is a fast and simple technique for identification of DNA sequence variations. It allows identifying single nucleotide differences by detecting minor changes in qPCR melting curves.
highQu ORA™ HRM qPCR Mix includes a proprietary intercalating saturating dye showing no inhibition for PCR. The dye has the same affinity for both AT or GC rich sequences what leads to highest accuracy in genotyping.

The hot-start function in the mix is based on the small molecular inhibitor technology and allows achieving highest sensitivity and specificity under both standard and fast qPCR cycling conditions. The mix provides excellent performance on both AT and GC rich templates and reliable results with minimum or no optimization.

COMPATIBILE INSTRUMENTS

Agilent:	AriaMx
BioRad:	CFX96™, CFX384™
Eppendorf:	Mastercycler® ep realplex Mastercycler® realplex 2S
Illumina/ PCRmax:	Eco
QIAGEN:	Rotor-Gene®Q, Rotor-Gene® 6000, Rotor-Gene® 3000
Roche Applied Science:	LightCycler®480, LightCycler®96, LightCycler®Nano
Thermo Fisher Scientific:	StepOne™, StepOnePlus™, 7500 FAST, 7900HT FAST, Viia7™, QuantStudio™ 6 / 7/ 12K Flex

PROTOCOL

- Use special primer selection programs for good planning.
- Work with amplicons in a range of 80-200, max 400 bp.
- Take typical measures to prevent PCR cross over contamination, keep your bench clean, wear gloves, use sterile tubes and filter pipet tips.
- Run reactions in triplets; include a no-template control and positive control in parallel.
- Thaw and keep reagents on ice. Mix well before use.
- Do not perform annealing/extension for more than 30 seconds and do not use lower than 60 °C temperature for this step.

IN VITRO RESEARCH USE ONLY

✓ Prepare a 20 µl reaction:

Reverse Primer	100-400 nM final c.
Forward Primer	100-400 nM final c.
cDNA Template or gDNA Template	<100 ng or 1 µg
PCR Water	to 10 µl
ORA™ HRM Mix, 2X	10 µl

✓ Mix gently, avoid bubbles.
✓ Place into the instrument (SYBR® Green or FAM channel) set like:

Initial denaturation	1 cycle: 95°C - 2 min for cDNA, or 1 cycle: 95°C - 3 min for gDNA
Denaturation	40 cycles: 95°C - 5 sec
Annealing/extension	40 cycles: 60 - 65°C – 20 - 30 sec

✓ Follow instrument instructions for melting curve analysis.

highQu RT qPCR kits are valued for their robust performance and ease of use with minimum optimization required. Supplied with PCR water, optimized for both common and fast cycling workflows, they convince by their early Ct values and by reproducible results they provide.

Want to try one? Order a sample today at www.highqu.com/Samples
Bulk orders are welcome at info@highQu.com.

One Step RT qPCR Kits

One Step RT qPCR Selection: Instrument Compatibility for Probe and Dye-based 1Step RT qPCR Kits

Probe-based One-step RT qPCR				Green dye-based One-step RT qPCR	
(2X or 4X) 1Step RT qPCR Probe	(2X or 4X) 1Step RT qPCR Probe ROX L	(2X or 4X) 1Step RT qPCR Probe ROX H	Instruments	1Step RT qPCR Green ROX L	1Step RT qPCR Green ROX H
page 29-30	*page 31-32*	*page 33-34*		*page 35*	*page 36*
●	●		**Analytic Jena**: qTOWER, qTOWER 2.x **BioRad**: Opticon®, Opticon®2, Chromo4™, MiniOpticon™, CFX96™, CFX384™ **Cepheid**: SmartCycler® **BJS**: Xxpress® **Illumina / PCRmax**: Eco **Eppendorf**: Mastercycler® ep realplex, Mastercycler® realplex 2S **Hain Lifescience**: FluoroCycler®96 **IT-IS Life Science**: MyGo Pro, MyGo Mini **QIAGEN**: Rotor-Gene®Q, Rotor-Gene® 6000, Rotor-Gene® 3000 **Roche Applied Science**: LightCycler®480, LightCycler®96, LightCycler®Nano **Takara**: Thermal Cycler Dice® **Thermo Fisher Scientific**: Piko Real® **Techne**: PrimeQ, Quantica®	●	
	●		**Agilent**: AriaMX, Mx3000P®, Mx3005P®, Mx4000P® **Fluidigm**: BioMark™ **Thermo Fisher Scientific**: 7500, 7500 FAST, Viia™7, QuantStudio™ 3 / 5 / 6 / 7 / 12K Flex	●	
		●	**Life Technologies**: 7000, 7300, 7700, 7900, 7900HT, 7900HT FAST, StepOne™, StepOnePlus™		●
●			**BioRad**: iCycler®, MyiQ™, iQ™5		

Info Tel: +497250 33 13 401 info@highQu.com

1Step RT qPCR Probe Kit

bulk quantities available

CAT.#	SIZE	COMPONENTS	COMPONENT COMPOSITION
QOP0101	200 r of 20 µl	2 x 1 ml - 1Step RT qPCR Probe Mix, 2X 2 x 0.2 ml - RT3 Mix, 20X (RNase Inhibitor+RTase) 2 x 1 ml - PCR Water	Hot Start Taq, dNTPs at 0.25 mM, optimized buffer 20X concentrated blend of modified MMuLV RT and RNase Inhibitor.
QOP0105	1000 r of 20 µl	10 x 1 ml - 1Step RT qPCR Probe Mix, 2X 10 x 0.2 ml - RT3 Mix, 20X (RNase Inhibitor+RTase) 10 x 1 ml - PCR Water	

Storage: *In the dark at -20°C.*

APPLICATIONS

- RT qPCR assays based on specific probes: including TaqMan®, Molecular Beacons, Scorpions™ Probes
- Quantification of any RNA template (mRNA, total RNA, viral RNA), low copy number genes

BENEFITS

- Reverse transcription & qPCR in one tube with highest sensitivity
- Efficient cDNA synthesis ensured by the thermostable Reverse Transcriptase & advanced RNase Inhibitor blend
- Universal Kit for standard and fast cycling, GC/AT rich templates
- Rapid extension, early Ct
- Supplied with PCR Water for maximum convenience

PRODUCT DETAILS

highQu 1Step RT qPCR mastermixes in combination with a blend of thermostable and extremely active Reverse Transcriptase & advanced RNase Inhibitor (RT Mix) allow for a single step one tube RT qPCR.
qPCR mastermixes are based on the small molecular inhibitor technology Hot Start PCR allowing to achieve highest sensitivity and specificity under both standard and fast qPCR cycling conditions. They provide excellent results on both AT and GC rich templates and guaranty rapid extension with early Ct values with minimum or no optimization.
To suit the broad instrument range the 1Step RT qPCR Probe Mixes are available in three versions – without ROX, with low or high ROX concentration. *See the selection table on page 28.*

PRECAUTIONS FOR WORK WITH RNA

Take care to prevent RNA from degradation by widely spread and stable RNases. Prepare crude samples and set up reactions in different dedicated areas, use DEPC-treated nuclease-free labware and gloves.

Before the cDNA synthesis, check RNA quality on denaturing agarose gel to be sure you have good quality material.

PROTOCOL

- Use special primer selection programs for good planning.
- Work with amplicons in a range of 80-200 bp, max 400 bp
- Take typical measures to prevent PCR cross over contamination, keep your bench clean, wear gloves, use sterile tubes and filter pipet tips.
- Run reactions in triplets; include a no-template control, no RT Mix control and positive control in parallel.
- Thaw and keep reagents on ice. Mix well before use.
- Higher amounts of RT3 Mix improve Ct, but primer dimers may appear.
- Do not perform annealing/extension for more than 30 seconds and do not use lower than 60 °C temperature for this step.

IN VITRO RESEARCH USE ONLY

✓ Prepare a 20 µl reaction:

Reverse Primer	100-400 nM final concentration
Forward Primer	100-400 nM final concentration
Specific Probe	200 nM final c. (0.4 µl of 10 µM)
Total RNA Template or mRNA Template	1 pg to 1 µg or >0.01 pg
PCR Water	to 10 µl
1Step RT qPCR Mix, 2X	10 µl
RT3 Mix, 20X	1 - 2 µl

✓ Mix gently, avoid bubbles.
✓ Place into the instrument set like:

Reverse Transcription	1 cycle: 40 - 55 °C – 10 min
Initial denaturation	1 cycles: 95°C - 2 min
Denaturation	40 cycles: 95°C - 5 sec
Annealing/extension	40 cycles: 60 - 65°C – 20 - 30 sec

✓ Follow instrument instructions for melting curve analysis.

For optional use, the ROX passive reference dye is premixed within the ROX L and ROX H qPCR Mixes. If the purchaser has an instrument capable of optional ROX detection and wishes to perform the optional normalization of the signal, then the user must select the option in the software.
Notice to Purchaser: With purchasing of this product, no rights are conveyed with respect to U.S. Patent: 5,928,907 and corresponding patents outside the US.

4X 1Step RT qPCR Probe Kit

bulk quantities available

CAT.#	SIZE	COMPONENTS	COMPONENT COMPOSITION
QOP0401	200 r of 20 µl	1 ml - 1Step RT qPCR Probe Mix, 4X 0.2 ml - RT4 Mix, 20X (RNase Inhibitor+RTase) 1 ml - PCR Water	1Step RT qPCR Probe Mix, 4X - contains Hot Start Taq, dNTPs, optimized buffer. RT4 Mix, 20X (RNase Inhibitor+RTase) is a 20X concentrated blend of modified MMuLV RT and RNase Inhibitor.
QOP0405	1000 r of 20 µl	5 x 1 ml - 1Step RT qPCR Probe Mix, 4X 5 x 0.2 ml - RT4 Mix, 20X (RNase Inhibitor+RTase) 5 x 1 ml - PCR Water	

Storage: *In the dark at -20°C.*

APPLICATIONS

- Viral RNA detection in diluted low copy number samples
- RT qPCR assays based on specific probes: including TaqMan®, Molecular Beacons, Scorpions™ Probes
- Quantification of any RNA template (mRNA, total RNA, viral RNA), low copy number genes

BENEFITS

- Robust 4X qPCR mix for high sample volume input up to 10 µl
- Detects <5 RNA copies per reaction
- Successfully tested for Sars-CoV-2 detection
- Reverse transcription and qPCR in one tube
- Ideal for multiplex reactions
- Universal - both standard and fast cycling, GC/AT rich templates

PRODUCT DETAILS

4X 1Step RT qPCR Probe Kits are designed for a sensitive detection of specific RNAs, including virus RNA, in diluted high-volume samples. They combine a robust 4X qPCR mix with a 20X blend of thermostable Reverse Transcriptase and RNase Inhibitor. This formulation allows for a high sample input volume with a reliable outcome of a single step RT qPCR when working with low copy number samples below 5 copies per reaction. 4X 1Step RT qPCR Probe Kits ensure robust performance of both reverse transcription and qPCR reactions, what allows for the highest sensitivity viral RNA detection under fast qPCR cycling conditions. The performance of the kits has been tested for Sars-CoV-2 detection in human specimens according to recommended Charité Berlin protocol with appropriate primers/probes.

PCR Water supplied in the kit ensures the best performance and reproducibility of the results.
Depending on your instrument requirements, the kit is available as no ROX, ROX L (low) and ROX H (high) versions.
See the selection table on page 28.

PRECAUTIONS FOR WORK WITH RNA

Take care to prevent RNA from degradation by widely spread and stable RNases. Prepare crude samples and set up reactions in different dedicated areas, use DEPC-treated nuclease-free labware and gloves.
Before the cDNA synthesis, check RNA quality on denaturing agarose gel to be sure you have good quality material.

PROTOCOL

- Use special primer selection programs for good planning.
- Work with amplicons in a range of 80-200 bp, max 400 bp.
- Take typical measures to prevent PCR cross over contamination, keep your bench clean, wear gloves, use sterile tubes and filter pipet tips.
- For controls, run reactions in triplets; include a no-template control, no RT Mix control and positive control in parallel.
- Thaw and keep reagents on ice. Mix them well before use!
- Use 2-5 or more microliters of swab extract for 20 µl reaction.
- 5 minutes are enough for reverse transcription at 45-55°C. For multiplex reactions 10 min RT step might be required.
- Do not perform annealing/extension for more than 30 seconds. Use 58 °C temperature for this step. Optimization between 58 and 65°C is possible if needed.

IN VITRO RESEARCH USE ONLY

✓ Prepare a 20 µl reaction:

Reverse Primer	0.5 – 1 µM final concentration
Forward Primer	0.5 – 1 µM final concentration
Specific Probe	150 – 500 nM final concentration
1Step RT qPCR Mix, 4X	5 µl
RT4 Mix, 20X	1 µl
Template (extracted RNA or crude sample from swabs)	2 – 5 µl (5 - 1x10^6 copies; you can add up to 7 - 10 µl in case of diluted RNA samples)
PCR Water	to 20 µl

✓ Mix gently, avoid bubbles.
✓ Place into the instrument set like:

Reverse Transcription	1 cycle: 50°C (45-55°C) - 5 min
Initial denaturation	1 cycles: 95°C - 3 min
Denaturation	50 cycles: 95°C - 15 sec.
Annealing/extension	50 cycles: 58°C (58-65°C) - 30 sec.

✓ Follow instrument instructions for melting curve analysis.

For optional use, the ROX passive reference dye is premixed within the ROX L and ROX H qPCR Mixes. If the purchaser has an instrument capable of optional ROX detection and wishes to perform the optional normalization of the signal, then the user must select the option in the software.
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1Step RT qPCR Probe ROX L Kit

bulk quantities available

CAT.#	SIZE	COMPONENTS	COMPONENT COMPOSITION
QOP0201	200 r of 20 µl	2 x 1 ml - 1Step RT qPCR Probe ROX L Mix, 2X 2 x 0.2 ml - RT3 Mix, 20X (RNase Inhibitor+RTase) 2 x 1 ml - PCR Water	Hot Start Taq, dNTPs at 0.25 mM, optimized buffer, low ROX concentration. 20X concentrated blend of modified MMuLV RT and RNase Inhibitor.
QOP0205	1000 r of 20 µl	10 x 1 ml - 1Step RT qPCR Probe ROX L Mix, 2X 10 x 0.2 ml - RT3 Mix, 20X (RNase Inhibitor+RTase) 10 x 1 ml - PCR Water	

Storage: *In the dark at -20°C.*

APPLICATIONS

- qPCR on instruments calibrated with low ROX conc.
- RT qPCR assays based on specific probes: including TaqMan®, Molecular Beacons, Scorpions™ Probes
- Quantification of any RNA template (mRNA, total RNA, viral RNA), low copy number genes

BENEFITS

- Reverse transcription & qPCR in one tube with highest sensitivity
- Efficient cDNA synthesis ensured by the thermostable Reverse Transcriptase & advanced RNase Inhibitor blend
- Universal Kit for standard and fast cycling, GC/AT rich templates
- Rapid extension, early Ct
- PCR Water supplied for maximum convenience

PRODUCT DETAILS

highQu 1Step RT qPCR mastermixes in combination with a blend of thermostable and extremely active Reverse Transcriptase & advanced RNase Inhibitor (RT Mix) allow for a single step one tube RT qPCR.
qPCR mastermixes are based on the small molecular inhibitor technology Hot Start PCR allowing to achieve highest sensitivity and specificity under both standard and fast qPCR cycling conditions. They provide excellent results on both AT and GC rich templates and guaranty rapid extension with early Ct values with minimum optimization.
To suit the broad instrument range the 1Step RT qPCR Probe Mixes are available in three versions – without ROX, with low or high ROX concentration. *See the selection table on page 28.*

PRECAUTIONS FOR WORK WITH RNA

Take care to prevent RNA from degradation by widely spread and stable RNases. Prepare crude samples and set up reactions in different dedicated areas, use DEPC-treated nuclease-free labware and gloves.

Before the cDNA synthesis, check RNA quality on denaturing agarose gel to be sure you have good quality material.

PROTOCOL

- Use special primer selection programs for good planning.
- Work with amplicons in a range of 80-200 bp, max 400 bp.
- Take typical measures to prevent PCR cross over contamination, keep your bench clean, wear gloves, use sterile tubes and filter pipet tips.
- Run reactions in triplets; include a no-template control, no RT Mix control and positive control in parallel.
- Thaw and keep reagents on ice. Mix well before use.
- Higher amounts of RT3 Mix improve Ct, but primer dimers may appear.
- Do not perform annealing/extension for more than 30 seconds and do not use lower than 60 °C temperature for this step.

IN VITRO RESEARCH USE ONLY

✓ Prepare a 20 µl reaction:

Reverse Primer	100-400 nM final concentration
Forward Primer	100-400 nM final concentration
Specific Probe	200 nM final c. (0.4 µl of 10 µM)
Total RNA Template or mRNA Template	1 pg to 1 µg or >0.01 pg
PCR Water	to 10 µl
1Step RT qPCR Mix, 2X	10 µl
RT3 Mix, 20X	1 - 2 µl

✓ Mix gently, avoid bubbles.
✓ Place into the instrument set like:

Reverse Transcription	1 cycle: 40 - 55 °C – 10 min
Initial denaturation	1 cycles: 95°C - 2 min
Denaturation	40 cycles: 95°C - 5 sec
Annealing/extension	40 cycles: 60 - 65°C – 20 - 30 sec

✓ Follow instrument instructions for melting curve analysis.

For optional use, the ROX passive reference dye is premixed within the ROX L and ROX H qPCR Mixes. If the purchaser has an instrument capable of optional ROX detection and wishes to perform the optional normalization of the signal, then the user must select the option in the software.
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4X 1Step RT qPCR Probe ROX L Kit

bulk quantities available

CAT.#	SIZE	COMPONENTS	COMPONENT COMPOSITION
QOP0501	200 r of 20 µl	1 ml - 1Step RT qPCR Probe ROX L Mix, 4X 0.2 ml - RT4 Mix, 20X (RNase Inhibitor+RTase) 1 ml - PCR Water	1Step RT qPCR Probe Mix, 4X - contains Hot Start Taq, dNTPs, optimized buffer, and low ROX concentration. RT4 Mix, 20X (RNase Inhibitor+RTase) - is a 20X concentrated blend of modified MMuLV RT and RNase Inhibitor.
QOP0505	1000 r of 20 µl	5 x 1 ml - 1Step RT qPCR Probe ROX L Mix, 4X 5 x 0.2 ml - RT4 Mix, 20X (RNase Inhibitor+RTase) 5 x 1 ml - PCR Water	

Storage: *In the dark at -20°C.*

APPLICATIONS

- Viral RNA detection in diluted low copy number samples
- RT qPCR assays based on specific probes: including TaqMan®, Molecular Beacons, Scorpions™ Probes
- Quantification of any RNA template (mRNA, total RNA, viral RNA), low copy number genes

BENEFITS

- Robust 4X qPCR mix for high sample volume input up to 10 µl
- Detects <5 RNA copies per reaction
- Successfully tested for Sars-CoV-2 detection
- Reverse transcription and qPCR in one tube
- Ideal for multiplex reactions
- Universal - both standard and fast cycling, GC/AT rich templates

PRODUCT DETAILS

4X 1Step RT qPCR Probe Kits are designed for a sensitive detection of specific RNAs, including virus RNA, in diluted high-volume samples. They combine a robust 4X qPCR mix with a 20X blend of thermostable Reverse Transcriptase and RNase Inhibitor. This formulation allows for a high sample input volume with a reliable outcome of a single step RT qPCR when working with low copy number samples below 5 copies per reaction. 4X 1Step RT qPCR Probe Kits ensure robust performance of both reverse transcription and qPCR reactions, what allows for the highest sensitivity viral RNA detection under fast qPCR cycling conditions. The performance of the kits has been tested for Sars-CoV-2 detection in human specimens according to recommended Charité Berlin protocol with appropriate primers/probes.

PCR Water supplied in the kit ensures the best performance and reproducibility of the results.
Depending on your instrument requirements, the kit is available as no ROX, ROX L (low) and ROX H (high) versions.
See the selection table on page 28.

PRECAUTIONS FOR WORK WITH RNA

Take care to prevent RNA from degradation by widely spread and stable RNases. Prepare crude samples and set up reactions in different dedicated areas, use DEPC-treated nuclease-free labware and gloves.
Before the cDNA synthesis, check RNA quality on denaturing agarose gel to be sure you have good quality material.

PROTOCOL

- Use special primer selection programs for good planning.
- Work with amplicons in a range of 80-200 bp, max 400 bp.
- Take typical measures to prevent PCR cross over contamination, keep your bench clean, wear gloves, use sterile tubes and filter pipet tips.
- For controls, run reactions in triplets; include a no-template control, no RT Mix control and positive control in parallel.
- Thaw and keep reagents on ice. Mix them well before use!
- Use 2-5 or more microliters of swab extract for 20 µl reaction.
- 5 minutes are enough for reverse transcription at 45-55°C. For multiplex reactions 10 min RT step might be required.
- Do not perform annealing/extension for more than 30 seconds. Use 58 °C temperature for this step. Optimization between 58 and 65°C is possible if needed.

IN VITRO RESEARCH USE ONLY

✓ Prepare a 20 µl reaction:

Reverse Primer	0.5 – 1 µM final concentration
Forward Primer	0.5 – 1 µM final concentration
Specific Probe	150 – 500 nM final concentration
1Step RT qPCR Mix, 4X	5 µl
RT4 Mix, 20X	1 µl
Template (extracted RNA or crude sample from swabs)	2 – 5 µl (5 - 1x10^6 copies; you can add up to 7 - 10 µl in case of diluted RNA samples)
PCR Water	to 20 µl

✓ Mix gently, avoid bubbles.
✓ Place into the instrument set like:

Reverse Transcription	1 cycle: 50°C (45-55°C) - 5 min
Initial denaturation	1 cycles: 95°C - 3 min
Denaturation	50 cycles: 95°C - 15 sec.
Annealing/extension	50 cycles: 58°C (58-65°C) - 30 sec.

✓ Follow instrument instructions for melting curve analysis.

For optional use, the ROX passive reference dye is premixed within the ROX L and ROX H qPCR Mixes. If the purchaser has an instrument capable of optional ROX detection and wishes to perform the optional normalization of the signal, then the user must select the option in the software.
Notice to Purchaser: With purchasing of this product, no rights are conveyed with respect to U.S. Patent: 5,928,907 and corresponding patents outside the US.

1Step RT qPCR Probe ROX H Kit

bulk quantities available

CAT.#	SIZE	COMPONENTS	COMPONENT COMPOSITION
QOP0301	200 r of 20 µl	2 x 1 ml - 1Step RT qPCR Probe ROX H Mix, 2X 2 x 0.2 ml - RT3 Mix, 20X (RNase Inhibitor+RTase) 2 x 1 ml - PCR Water	Hot Start Taq, dNTPs at 0.25 mM, optimized buffer, high ROX concentration. 20X concentrated blend of modified MMuLV RT and RNase Inhibitor.
QOP0305	1000 r of 20 µl	10 x 1 ml - 1Step RT qPCR Probe ROX H Mix, 2X 10 x 0.2 ml - RT3 Mix, 20X (RNase Inhibitor+RTase) 10 x 1 ml - PCR Water	

Storage: *In the dark at -20°C.*

APPLICATIONS

- qPCR on instruments calibrated with high ROX conc.
- RT qPCR assays based on specific probes: including TaqMan®, Molecular Beacons, Scorpions™ Probes
- Quantification of any RNA template (mRNA, total RNA, viral RNA), low copy number genes

BENEFITS

- Reverse transcription & qPCR in one tube with highest sensitivity
- Efficient cDNA synthesis ensured by the thermostable Reverse Transcriptase & advanced RNase Inhibitor blend
- Universal Kit for standard and fast cycling, GC/AT rich templates
- Rapid extension, early Ct
- PCR Water supplied for maximum convenience

PRODUCT DETAILS

highQu 1Step RT qPCR mastermixes in combination with a blend of thermostable and extremely active Reverse Transcriptase & advanced RNase Inhibitor (RT Mix) allow for a single step one tube RT qPCR.
qPCR mastermixes are based on the small molecular inhibitor technology Hot Start PCR allowing to achieve highest sensitivity and specificity under both standard and fast qPCR cycling conditions. They provide excellent results on both AT and GC rich templates and guaranty rapid extension with early Ct values with minimum optimization.
To suit the broad instrument range the 1Step RT qPCR Probe Mixes are available in three versions – without ROX, with low or high ROX concentration. *See the selection table on page 28.*

PRECAUTIONS FOR WORK WITH RNA

Take care to prevent RNA from degradation by widely spread and stable RNases. Prepare crude samples and set up reactions in different dedicated areas, use DEPC-treated nuclease-free labware and gloves.

Before the cDNA synthesis, check RNA quality on denaturing agarose gel to be sure you have good quality material.

PROTOCOL

- Use special primer selection programs for good planning.
- Work with amplicons in a range of 80-200 bp, max 400 bp.
- Take typical measures to prevent PCR cross over contamination, keep your bench clean, wear gloves, use sterile tubes and filter pipet tips.
- Run reactions in triplets; include a no-template control, no RT Mix control and positive control in parallel.
- Thaw and keep reagents on ice. Mix well before use.
- Higher amounts of RT3 Mix improve Ct, but primer dimers may appear.
- Do not perform annealing/extension for more than 30 seconds and do not use lower than 60 °C temperature for this step.

IN VITRO RESEARCH USE ONLY

✓ Prepare a 20 µl reaction:

Reverse Primer	100-400 nM final concentration
Forward Primer	100-400 nM final concentration
Specific Probe	200 nM final c. (0.4 µl of 10 µM)
Total RNA Template or mRNA Template	1 pg to 1 µg or >0.01 pg
PCR Water	to 10 µl
1Step RT qPCR Mix, 2X	10 µl
RT3 Mix, 20X	1 - 2 µl

✓ Mix gently, avoid bubbles.
✓ Place into the instrument set like:

Reverse Transcription	1 cycle: 40 - 55 °C – 10 min
Initial denaturation	1 cycles: 95°C - 2 min
Denaturation	40 cycles: 95°C - 5 sec
Annealing/extension	40 cycles: 60 - 65°C – 20 - 30 sec

✓ Follow instrument instructions for melting curve analysis.

For optional use, the ROX passive reference dye is premixed within the ROX L and ROX H qPCR Mixes. If the purchaser has an instrument capable of optional ROX detection and wishes to perform the optional normalization of the signal, then the user must select the option in the software.
Notice to Purchaser: With purchasing of this product, no rights are conveyed with respect to U.S. Patent: 5,928,907 and corresponding patents outside the US.

4X 1Step RT qPCR Probe ROX H Kit

bulk quantities available

CAT.#	SIZE	COMPONENTS	COMPONENT COMPOSITION
QOP0601	200 r of 20 µl	1 ml - 1Step RT qPCR Probe ROX H Mix, 4X 0.2 ml - RT4 Mix, 20X (RNase Inhibitor+RTase) 1 ml - PCR Water	1Step RT qPCR Probe Mix, 4X - contains Hot Start Taq, dNTPs, optimized buffer, and high ROX concentration. RT4 Mix, 20X (RNase Inhibitor+RTase) - is a 20X concentrated blend of modified MMuLV RT and RNase Inhibitor.
QOP0605	1000 r of 20 µl	5 x 1 ml - 1Step RT qPCR Probe ROX H Mix, 2X 5 x 0.2 ml - RT4 Mix, 20X (RNase Inhibitor+RTase) 5 x 1 ml - PCR Water	

Storage: *In the dark at -20°C.*

APPLICATIONS

- Viral RNA detection in diluted low copy number samples
- RT qPCR assays based on specific probes: including TaqMan®, Molecular Beacons, Scorpions™ Probes
- Quantification of any RNA template (mRNA, total RNA, viral RNA), low copy number genes

BENEFITS

- Robust 4X qPCR mix for high sample volume input up to 10 µl
- Detects <5 RNA copies per reaction
- Successfully tested for Sars-CoV-2 detection
- Reverse transcription and qPCR in one tube
- Ideal for multiplex reactions
- Universal - both standard and fast cycling, GC/AT rich templates

PRODUCT DETAILS

4X 1Step RT qPCR Probe Kits are designed for a sensitive detection of specific RNAs, including virus RNA, in diluted high-volume samples. They combine a robust 4X qPCR mix with a 20X blend of thermostable Reverse Transcriptase and RNase Inhibitor. This formulation allows for a high sample input volume with a reliable outcome of a single step RT qPCR when working with low copy number samples below 5 copies per reaction. 4X 1Step RT qPCR Probe Kits ensure robust performance of both reverse transcription and qPCR reactions, what allows for the highest sensitivity viral RNA detection under fast qPCR cycling conditions. The performance of the kits has been tested for Sars-CoV-2 detection in human specimens according to recommended Charité Berlin protocol with appropriate primers/probes.

PCR Water supplied in the kit ensures the best performance and reproducibility of the results.
Depending on your instrument requirements, the kit is available as no ROX, ROX L (low) and ROX H (high) versions.
See the selection table on page 28.

PRECAUTIONS FOR WORK WITH RNA

Take care to prevent RNA from degradation by widely spread and stable RNases. Prepare crude samples and set up reactions in different dedicated areas, use DEPC-treated nuclease-free labware and gloves.
Before the cDNA synthesis, check RNA quality on denaturing agarose gel to be sure you have good quality material.

PROTOCOL

- Use special primer selection programs for good planning.
- Work with amplicons in a range of 80-200 bp, max 400 bp.
- Take typical measures to prevent PCR cross over contamination, keep your bench clean, wear gloves, use sterile tubes and filter pipet tips.
- For controls, run reactions in triplets; include a no-template control, no RT Mix control and positive control in parallel.
- Thaw and keep reagents on ice. Mix them well before use!
- Use 2-5 or more microliters of swab extract for 20 µl reaction.
- 5 minutes are enough for reverse transcription at 45-55°C. For multiplex reactions 10 min RT step might be required.
- Do not perform annealing/extension for more than 30 seconds. Use 58 °C temperature for this step. Optimization between 58 and 65°C is possible if needed.

IN VITRO RESEARCH USE ONLY

✓ Prepare a 20 µl reaction:

Reverse Primer	0.5 – 1 µM final concentration
Forward Primer	0.5 – 1 µM final concentration
Specific Probe	150 – 500 nM final concentration
1Step RT qPCR Mix, 4X	5 µl
RT4 Mix, 20X	1 µl
Template (extracted RNA or crude sample from swabs)	2 – 5 µl (5 - 1x10^6 copies; you can add up to 7 - 10 µl in case of diluted RNA samples)
PCR Water	to 20 µl

✓ Mix gently, avoid bubbles.
✓ Place into the instrument set like:

Reverse Transcription	1 cycle: 50°C (45-55°C) - 5 min
Initial denaturation	1 cycles: 95°C - 3 min
Denaturation	50 cycles: 95°C - 15 sec.
Annealing/extension	50 cycles: 58°C (58-65°C) - 30 sec.

✓ Follow instrument instructions for melting curve analysis.

For optional use, the ROX passive reference dye is premixed within the ROX L and ROX H qPCR Mixes. If the purchaser has an instrument capable of optional ROX detection and wishes to perform the optional normalization of the signal, then the user must select the option in the software.
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1Step RT qPCR Green ROX L Kit

bulk quantities available

CAT.#	SIZE	COMPONENTS	COMPONENT COMPOSITION
QOD0101	200 r of 20 µl	2 x 1 ml - 1Step RT qPCR Green ROX L Mix, 2X 2 x 0.2 ml - RT3 Mix, 20X (RNase Inhibitor+RTase) 2 x 1 ml - PCR Water	Hot Start Taq, dNTPs at 0.25 mM, optimized buffer, low ROX concentration. 20X concentrated blend of modified MMuLV RT and RNase Inhibitor.
QOD0105	1000 r of 20 µl	10 x 1 ml - 1Step RT qPCR Green ROX L Mix, 2X 10 x 0.2 ml - RT3 Mix, 20X (RNase Inhibitor+RTase) 10 x 1 ml - PCR Water	

Storage: *In the dark at -20°C.*

APPLICATIONS

- Relative gene expression analysis, absolute quantification of any RNA template (mRNA, total RNA, viral RNA), low copy number genes
- Intercalating dye fluorescence based qPCR on instruments calibrated with low ROX conc.

BENEFITS

- Reverse transcription & qPCR in one tube with highest sensitivity
- Efficient cDNA synthesis ensured by the thermostable Reverse Transcriptase & advanced RNase Inhibitor blend
- Universal Kit for standard and fast cycling, GC/AT rich templates
- Rapid extension, early Ct
- PCR Water supplied for maximum convenience

PRODUCT DETAILS

highQu 1Step RT qPCR mastermixes in combination with a blend of thermostable and extremely active Reverse Transcriptase & advanced RNase Inhibitor (RT Mix) allow for a single step one tube RT qPCR.
qPCR mastermixes are based on the small molecular inhibitor technology Hot Start PCR allowing to achieve highest sensitivity and specificity under both standard and fast qPCR cycling conditions. They provide excellent results on both AT and GC rich templates and guaranty rapid extension with early Ct values with minimum or no optimization.
To suit the broad instrument range the 1Step RT qPCR Green Mixes are available in different versions – with low or high ROX concentration. *See the selection table on page 28.*

PRECAUTIONS FOR WORK WITH RNA

Take care to prevent RNA from degradation by widely spread and stable RNases. Prepare crude samples and set up reactions in different dedicated areas, use DEPC-treated nuclease-free labware and gloves.

Before the cDNA synthesis, check RNA quality on denaturing agarose gel to be sure you have good quality material.

PROTOCOL

- Use special primer selection programs for good planning.
- Work with amplicons in a range of 80-200 bp, max 400 bp.
- Take typical measures to prevent PCR cross over contamination, keep your bench clean, wear gloves, use sterile tubes and filter pipet tips.
- Run reactions in triplets; include a no-template control, no RT Mix control and positive control in parallel.
- Thaw and keep reagents on ice. Mix well before use.
- Higher amounts of RT3 Mix improve Ct, but primer dimers may appear.
- Do not perform annealing/extension for more than 30 seconds and do not use lower than 60 °C temperature for this step.

IN VITRO RESEARCH USE ONLY

✓ Prepare a 20 µl reaction:

Reverse Primer	100-400 nM final concentration
Forward Primer	100-400 nM final concentration
Specific Probe	200 nM final c. (0.4 µl of 10 µM)
Total RNA Template or mRNA Template	1 pg to 1 µg or >0.01 pg
PCR Water	to 10 µl
1Step RT qPCR Mix, 2X	10 µl
RT3 Mix, 20X	1 - 2 µl

✓ Mix gently, avoid bubbles.
✓ Place into the instrument set like:

Reverse Transcription	1 cycle: 40 - 55 °C – 10 min
Initial denaturation	1 cycles: 95°C - 2 min
Denaturation	40 cycles: 95°C - 5 sec
Annealing/extension	40 cycles: 60 - 65°C – 20 - 30 sec

✓ Follow instrument instructions for melting curve analysis.

For optional use, the ROX passive reference dye is premixed within the ROX L and ROX H qPCR Mixes. If the purchaser has an instrument capable of optional ROX detection and wishes to perform the optional normalization of the signal, then the user must select the option in the software.
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1Step RT qPCR Green ROX H Kit

bulk quantities available

CAT.#	SIZE	COMPONENTS	COMPONENT COMPOSITION
QOD0201	200 r of 20 µl	2 x 1 ml - 1Step RT qPCR Green ROX H Mix, 2X 2 x 0.2 ml - RT3 Mix, 20X (RNase Inhibitor+RTase) 2 x 1 ml - PCR Water	Hot Start Taq, dNTPs at 0.25 mM, optimized buffer, high ROX concentration. 20X concentrated blend of modified MMuLV RT and RNase Inhibitor.
QOD0205	1000 r of 20 µl	10 x 1 ml - 1Step RT qPCR Green ROX H Mix, 2X 10 x 0.2 ml - RT3 Mix, 20X (RNase Inhibitor+RTase) 10 x 1 ml - PCR Water	

Storage: *In the dark at -20°C.*

APPLICATIONS

- Relative gene expression analysis, absolute quantification of any RNA template (mRNA, total RNA, viral RNA), low copy number genes
- Intercalating dye fluorescence based qPCR on instruments calibrated with high ROX conc.

BENEFITS

- Reverse transcription & qPCR in one tube with highest sensitivity
- Efficient cDNA synthesis ensured by the thermostable Reverse Transcriptase & advanced RNase Inhibitor blend
- Universal Kit for standard and fast cycling, GC or AT rich templates
- Rapid extension, early Ct
- PCR Water supplied for maximum convenience

PRODUCT DETAILS

highQu 1Step RT qPCR mastermixes in combination with a blend of thermostable and extremely active Reverse Transcriptase & advanced RNase Inhibitor (RT Mix) allow for a single step one tube RT qPCR.
qPCR mastermixes are based on the small molecular inhibitor technology Hot Start PCR allowing to achieve highest sensitivity and specificity under both standard and fast qPCR cycling conditions. They provide excellent results on both AT and GC rich templates and guaranty rapid extension with early Ct values with minimum or no optimization.
To suit the broad instrument range the 1Step RT qPCR Green Mixes are available in different versions – with low or high ROX concentration. *See the selection table on page 28.*

PRECAUTIONS FOR WORK WITH RNA

Take care to prevent RNA from degradation by widely spread and stable RNases. Prepare crude samples and set up reactions in different dedicated areas, use DEPC-treated nuclease-free labware and gloves.

Before the cDNA synthesis, check RNA quality on denaturing agarose gel to be sure you have good quality material.

PROTOCOL

- Use special primer selection programs for good planning.
- Work with amplicons in a range of 80-200 bp, max 400 bp.
- Take typical measures to prevent PCR cross over contamination, keep your bench clean, wear gloves, use sterile tubes and filter pipet tips.
- Run reactions in triplets; include a no-template control, no RT Mix control and positive control in parallel.
- Thaw and keep reagents on ice. Mix well before use.
- Higher amounts of RT3 Mix improve Ct, but primer dimers may appear.
- Do not perform annealing/extension for more than 30 seconds and do not use lower than 60 °C temperature for this step.

IN VITRO RESEARCH USE ONLY

✓ Prepare a 20 µl reaction:

Reverse Primer	100-400 nM final concentration
Forward Primer	100-400 nM final concentration
Specific Probe	200 nM final c. (0.4 µl of 10 µM)
Total RNA Template or mRNA Template	1 pg to 1 µg or >0.01 pg
PCR Water	to 10 µl
1Step RT qPCR Mix, 2X	10 µl
RT3 Mix, 20X	1 - 2 µl

✓ Mix gently, avoid bubbles.
✓ Place into the instrument set like:

Reverse Transcription	1 cycle: 40 - 55 °C – 10 min
Initial denaturation	1 cycles: 95°C - 2 min
Denaturation	40 cycles: 95°C - 5 sec
Annealing/extension	40 cycles: 60 - 65°C – 20 - 30 sec

✓ Follow instrument instructions for melting curve analysis.

For optional use, the ROX passive reference dye is premixed within the ROX L and ROX H qPCR Mixes. If the purchaser has an instrument capable of optional ROX detection and wishes to perform the optional normalization of the signal, then the user must select the option in the software.
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End-point PCR
Enzymes and Master Mixes

End-point PCR Selection: Routine, Hot-start, Direct, Long, High fidelity and Complex PCR

	Routine		Hot Start & Direct		Long, Complex	High Fidelity & Complex		
	ALLin™ Taq & Mixes	Taq DNA Poly-merase	ALLin™ HS Taq & Mixes	SampleIN™ Direct PCR Kit	ALLin™ RPH & Mix	ALLin™ HiFi	ALLin™ Mega HiFi & Mixes	ALLin™ Mega HS HiFi & Mixes
	page 39-41	*page 42*	*page 43-45*	*page 46*	*page 47-48*	*page 49*	*page 50-52*	*page 53-55*
Fast cycling	●		●	●	●	●	●●	●●
GC/AT rich PCR	●		●●	●●	●●	●●	●●●	●●●
Hot Start			●	●	●			●
High sensitivity			●	●	●			●
Fidelity vs Taq	1 X	1 X	1 X	1 X	5 X	50 X	100 X	100 X
Long PCR					●●	●	●●	●●
Max. amplicon	6 kb	5 kb	6 kb	6 kb	35 kb	10 kb	>20 kb	>20 kb
High yields	●●		●●	●	●	●	●●	●●●
Direct PCR	Colony	Colony	Colony, blood, urine	Mouse tail, ear, blood, tissues, hair, plant, swab	Colony, blood	Colony	Colony	Colony
Multiplex PCR	●		●●	●	●●		●	●●●
Cloning	TA	TA	TA	TA	TA	Blunt	Blunt	Blunt
Direct Loading on gels	with Red Mix		with Red Mix	●			with Red Mix	with Red Mix
ALLin™ Buffer with dNTPs*	●		●		●	●	●	●
Mastermix	Mix & Red Mix		Mix & Red Mix	Red Mix	Mix		Mix & Red Mix	Mix & Red Mix

* ALLin™ PCR buffers contain optimal dNTP and magnesium concentrations, PCR enhancers and stabilizers what allows for easier amplification of GC or AT rich templates and for success in fast cycling

ALLin™ Taq DNA Polymerase

CAT.#	SIZE	COMPONENTS	COMPONENT COMPOSITION
PCE0101	500 u	500 u - ALLin™ Taq DNA Polymerase, 5 u/µl 4 x 1 ml - 5X ALLin™ PCR Buffer	Enzyme in storage buffer. 1X ALLin™ PCR Buffer contains 0.25 mM dNTPs, 3 mM $MgCl_2$, enhancers, stabilizers.
PCE0105	2500 u	5 x 500 u - ALLin™ Taq DNA Polymerase, 5 u/µl 20 x 1 ml - 5X ALLin™ PCR Buffer	
Storage:	*In the dark at -20°C.*		

APPLICATIONS

- Routine PCR up to 6 kb
- Amplification of complex (GC/AT rich) templates
- Colony PCR
- Fast PCR
- TA cloning

BENEFITS

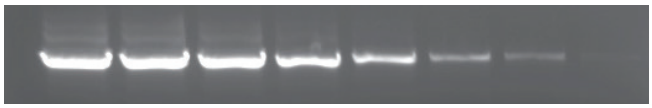
- Engineered Taq combined with advanced buffer - a synergy providing advantages over classical Taq Polymerases
- Higher yields under standard and fast cycling
- Increased success in amplification of longer templates (6 kb)
- Robust amplification of GC/AT rich templates
- 5X ALLin™ PCR Buffer contains optimal Mg2+ and dNTPs

PRODUCT DETAILS

highQu ALLin™ Taq DNA Polymerase is the versatile engineered enzyme which in combination with the optimized ALLin™ buffer provides higher success rates in demanding PCR applications like amplification of complex templates, crude sample PCR and fast cycling.

ALLin™ Taq DNA Polymerase has the same PCR accuracy like Taq DNA Polymerase, 4.5 x 10^4 (a number of correct nucleotides incorporated before the first error) and produces A-tailed products suitable for ligating into TA cloning vectors.
For the maximum convenience the 2X ALLin™ Red Taq Mastermix and 2X ALLin™ Taq Mastermix are available.
See next pages.

PERFORMANCE



highQu ALLin™ Taq DNA Polymerase (above) shows better yields and higher sensitivity compared to competitor Taq DNA Polymerase (below).
PCR of a 1.2 kb fragment of 60% GC GAPDH, from human genomic DNA, in a 3 fold dilution from left to right. Starting from 200 ng of DNA up to 0.7 pg in the 7th dilution.

PROTOCOL

- Take typical measures to prevent PCR cross over contamination, keep your bench clean, wear gloves, use sterile tubes and filter pipet tips.
- Include a no-template control and positive control in parallel.
- Thaw and keep reagents on ice. Mix well before use.
- The longer the amplicon, the longer the extension time: Use 15 sec/kb extension.
- Use 90 sec extension for multiplexing.
- Run an annealing temperature gradient from 55°C to 65°C to choose the best specificity conditions.
- Do not use fast cycling for multiplexing.

IN VITRO RESEARCH USE ONLY

✓ Prepare a 50 µl reaction:

Rev. & For. Primers	0.1-0.4 µM final each (≤2 µl of 10 µM)
cDNA Template or gDNA Template	< 100 ng or 5-500 ng
5X ALLin™ PCR Buffer	10 µl
Water (PCR Water, WAT0110)	to 49 µl
ALLin™ Taq DNA Polymerase, 5 u/µl	0.25 - 1 µl

✓ Mix gently, avoid bubbles.
✓ Place into the instrument set like:

Initial denaturation	1 cycle: 95°C - 1 min
Denaturation	40 cycles: 95°C - 15 sec
Annealing	40 cycles: 55-65°C – 15 sec
Extension	40 cycles: 72°C – 1-90 sec (15 sec/kb)

✓ Store probes for short time on ice, for long at -20°C.

ALLin™ Red Taq Mastermix, 2X

bulk quantities available

CAT.#	SIZE	COMPONENTS	COMPONENT COMPOSITION
PCM0201	200 r of 50 µl	5 x 1 ml - ALLin™ Red Taq Mastermix, 2X 5 x 1 ml - PCR Water	1X mastermix contains 0.25 mM dNTPs, 3 mM $MgCl_2$, enhancers, stabilizers, red electrophoresis tracking dye and density reagents for gel loading.
PCM0205	1000 r of 50 µl	25 x 1 ml - ALLin™ Red Taq Mastermix, 2X 25 x 1 ml - PCR Water	
Storage:	*In the dark at -20°C.*		

APPLICATIONS

- Routine PCR up to 6 kb for a direct gel loading
- Amplification of complex (GC/AT rich) templates
- Colony PCR
- Fast PCR
- TA cloning

BENEFITS

- Engineered Taq combined with advanced buffer - a synergy providing advantages over classical Taq Polymerases
- Premixed with red dye and density reagents for direct loading on the gels after the PCR
- Higher yields under standard and fast cycling
- Increased success in amplification of longer templates (6 kb)
- Robust amplification of GC/AT rich templates

PRODUCT DETAILS

highQu ALLin™ Taq DNA Polymerase is the versatile engineered enzyme which in combination with the optimized ALLin™ buffer provides higher success rates in demanding PCR applications like amplification of complex templates, crude sample PCR and fast cycling.

ALLin™ Taq DNA Polymerase has the same PCR accuracy like Taq DNA Polymerase, 4.5 x 10^4 (a number of correct nucleotides incorporated before the first error) and produces A-tailed products suitable for ligating into TA cloning vectors.

The convenience of ALLin™ Taq DNA Polymerase is maximized by the use of 2X Mastermix providing the additional advantage of reduced pipetting and minimized errors.
ALLin™ Red Taq Mastermix, 2X is premixed with red dye and density reagents for direct loading on the gels after the PCR.
In a 2% agarose TAE gel the dye migrates with~350 bp DNA, in 1% agarose TAE gel with ~ 600 bp DNA fragments.
The mastermix is even supplied with PCR water.

PROTOCOL

- Take typical measures to prevent PCR cross over contamination, keep your bench clean, wear gloves, use sterile tubes and filter pipet tips.
- Include a no-template control and positive control in parallel.
- Thaw and keep reagents on ice. Mix well before use.
- The longer the amplicon, the longer the extension time: Use 15 sec/kb extension.
- Use 90 sec extension for multiplexing. Do not use fast cycling for multiplexing.
- Run an annealing temperature gradient from 55°C to 65°C to choose the best specificity conditions.
- ALLin™ Red Taq Mastermix, 2X is premixed with red dye and density reagents for direct loading on the gels. In a 2% agarose TAE gel the dye migrates with~350 bp DNA, in 1% agarose TAE gel with ~ 600 bp DNA fragments.

IN VITRO RESEARCH USE ONLY

✓ Prepare a 50 µl reaction:

Rev. & For. Primers	0.1-0.4 µM final each (≤2 µl of 10 µM)
cDNA Template or gDNA Template	< 100 ng or 5 - 500 ng
PCR Water	to 25 µl
ALLin™ Red Taq Mastermix, 2X	25 µl

✓ Mix gently, avoid bubbles.
✓ Place into the instrument set like:

Initial denaturation	1 cycle: 95°C - 1 min
Denaturation	40 cycles: 95°C - 15 sec
Annealing	40 cycles: 55-65°C – 15 sec
Extension	40 cycles: 72°C – 1-90 sec (15 sec/kb)

✓ Load probes on the agarose gel. The red loading dye is included in the mastermix.
✓ Store probes for short time on ice, for long at -20°C.

ALLin™ Taq Mastermix, 2X

bulk quantities available

CAT.#	SIZE	COMPONENTS	COMPONENT COMPOSITION
PCM0101	200 r of 50 µl	5 x 1 ml - ALLin™ Taq Mastermix, 2X 5 x 1 ml - PCR Water	1X mastermix contains 0.25 mM dNTPs, 3 mM $MgCl_2$, enhancers, stabilizers.
PCM0105	1000 r of 50 µl	25 x 1 ml - ALLin™ Taq Mastermix, 2X 25 x 1 ml - PCR Water	
Storage:	*In the dark at -20°C.*		

APPLICATIONS

- Routine PCR up to 6 kb
- Amplification of complex (GC/AT rich) templates
- Colony PCR
- Fast PCR
- TA cloning

BENEFITS

- Engineered Taq combined with advanced buffer - a synergy providing advantages over classical Taq Polymerases
- Higher yields under standard and fast cycling
- Increased success in amplification of longer templates (6 kb)
- Robust amplification of GC/AT rich templates

PRODUCT DETAILS

highQu ALLin™ Taq DNA Polymerase is the versatile engineered enzyme which in combination with the optimized ALLin™ buffer provides higher success rates in demanding PCR applications like amplification of complex templates, crude sample PCR and fast cycling.

ALLin™ Taq DNA Polymerase has the same PCR accuracy like Taq DNA Polymerase, 4.5 x 10^4 (a number of correct nucleotides incorporated before the first error) and produces A-tailed products suitable for ligating into TA cloning vectors.

The convenience of ALLin™ Taq DNA Polymerase is maximized by the use of 2X Mastermix providing the additional advantage of reduced pipetting and minimized errors.

The mastermix is even supplied with PCR water.

PROTOCOL

- Take typical measures to prevent PCR cross over contamination, keep your bench clean, wear gloves, use sterile tubes and filter pipet tips.
- Include a no-template control and positive control in parallel.
- Thaw and keep reagents on ice. Mix well before use.
- The longer the amplicon, the longer the extension time: Use 15 sec/kb extension.
- Use 90 sec extension for multiplexing. Do not use fast cycling for multiplexing.
- Run an annealing temperature gradient from 55°C to 65°C to choose the best specificity conditions.

IN VITRO RESEARCH USE ONLY

✓ Prepare a 50 µl reaction:

Rev. & For. Primers	0.1-0.4 µM final each (≤2 µl of 10 µM)
cDNA Template or gDNA Template	< 100 ng or 5-500 ng
PCR Water	to 25 µl
ALLin™ Taq Mastermix, 2X	25 µl

✓ Mix gently, avoid bubbles.
✓ Place into the instrument set like:

Initial denaturation	1 cycle: 95°C - 1 min
Denaturation	40 cycles: 95°C - 15 sec
Annealing	40 cycles: 55-65°C – 15 sec
Extension	40 cycles: 72°C – 1-90 sec (15 sec/kb)

✓ Store probes for short time on ice, for long at -20°C.

Taq DNA Polymerase

CAT.#	SIZE	COMPONENTS	COMPONENT COMPOSITION
PCE0201	1500 u	1500 u - Taq DNA Polymerase, 5 u/µl 4 x 2 ml - 10X PCR Buffer 2 x 2 ml - 50 mM $MgCl_2$	Enzyme in storage buffer. 10X PCR Buffer contains enhancers and stabilizers, but no dNTPs and no Mg^{2+}.
PCE0202	3000 u	2 x 1500 u - Taq DNA Polymerase, 5 u/µl 8 x 2 ml - 10X PCR Buffer 4 x 2 ml - 50 mM $MgCl_2$	

Storage: *In the dark at -20°C.*

APPLICATIONS

- Routine PCR up to 5 kb
- RT-PCR, Colony PCR
- TA cloning, library construction
- Genotyping, screening

BENEFITS

- High yields in routine PCR, good performance in fast PCR
- Guarantied successful DNA preparation for TA cloning
- Robust on complex templates

PRODUCT DETAILS

highQu Taq DNA Polymerase is the classical enzyme for routine PCR applications providing high amplification yields of 3-5 kb targets under various conditions. Taq DNA Polymerase is purified from a recombinant *E. coli* strain carrying the Taq DNA polymerase gene. Taq DNA polymerase is thermostable 5´→ 3´ DNA polymerase. It lacks 3´→ 5´ exonuclease (proofreading) activity and has low 5´→ 3´ exonuclease activity. Polymerase exhibits deoxynucleotidyl transferase activity resulting in A-overhang at the 3'-ends of PCR products and allowing for TA cloning. The PCR accuracy of Taq DNA Polymerase is 4.5 x 10^4 (a number of nucleotides incorporated before the first error occurs).

RECOMMENDATIONS

The supplied reaction buffer contains no dNTPs and the 50 mM $MgCl_2$ is provided separately what allows for magnesium optimization upon the need. dNTPs in mixes or sets can be purchased separately (page 59)

- Typical concentration of each dNTP in the reaction is 0.2 – 0.25 mM. Higher concentration increase yields, however Mg^{2+}-ions bind to dNTPs, therefore, both components shall be present in coordinated concentrations. Too high dNTPs and magnesium concentrations reduce PCR fidelity.
- Mix well each dNTP and magnesium solution.
- Use final 3 mM $MgCl_2$ and 0.25 mM each dNTP concentrations for routine PCR.

Starting dNTP Mix conc.	Vol. of dNTP mix in 50 µl r.	Final Mg^{2+} conc. in r.	Vol. of 50 mM $MgCl_2$ in 50 µl rxn to achieve desired conc.
10 mM	1.25 µl	2 mM	2 µl
25 mM	0.5 µl	3 mM	3 µl

PROTOCOL

- Take typical measures to prevent PCR cross over contamination, keep your bench clean, wear gloves, use sterile tubes and filter pipet tips.
- Include a no-template control and positive control in parallel.
- Thaw and keep reagents on ice. Mix well before use.
- The longer the amplicon, the longer the extension time. Taq DNA Polymerase's speed is ~ 2000 nucleotides/min, so 15-90 seconds of extension can be provided per cycle, depending on amplicon size.
- Start annealing with 55°C and perform gradient by increasing temperature in 2°C up to 65°C to choose thebest. Calculate primer annealing temperature using software.

✓ Prepare a 50 µl reaction:

Rev. & For. Primers	0.1-0.5 µM final (1-2 µl of 10 µM each)
cDNA Template or gDNA Template	< 100 ng or 5 - 500 ng
10X PCR Buffer	5 µl
dNTP Mix (NUM0201)	0.25 mM final (1.25 µl of 10 mM dNTP mix)
50 mM Mg Cl_2	5 µl
Water (PCR Water, WAT0110)	to 49 µl
Taq DNA Polymerase, 5 u/µl	0.25 - 1 µl

✓ Mix gently, avoid bubbles. Place into the instrument set like:

Initial denaturation	1 cycle: 95°C - 60 sec
Denaturation	30-40 cycles: 95°C - 15 sec
Annealing	30-40 cycles: 55-65°C – 15 sec
Extension	30-40 cycles: 72 °C – 15-90 sec
Final Extension	1 cycle: 72°C – 5 min (for TA cloning)

✓ Store probes for short time on ice, for long at -20°C.

IN VITRO RESEARCH USE ONLY

ALLin™ Hot Start Taq Polymerase

bulk quantities available

CAT.#	SIZE	COMPONENTS	COMPONENT COMPOSITION
HSE0101	500 u	2x 250 u - ALLin™ Hot Start Taq Polymerase, 5 u/µl 4 x 1 ml - 5X ALLin™ PCR Buffer	Enzyme in storage buffer. 1X ALLin™ PCR Buffer contains 0.25 mM dNTPs, 3 mM $MgCl_2$, enhancers, stabilizers.
HSE0105	2500 u	10 x 250 u - ALLin™ Hot Start Taq Polymerase, 5 u/µl 20 x 1 ml - 5X ALLin™ PCR Buffer	
Storage:	*In the dark at -20°C.*		

APPLICATIONS

- Sensitive hot-start PCR up to 6 kb
- Low copy target detection
- Amplification of complex (GC/AT rich) templates
- Fast PCR
- TA cloning
- Multiplex hot-start PCR
- Colony PCR

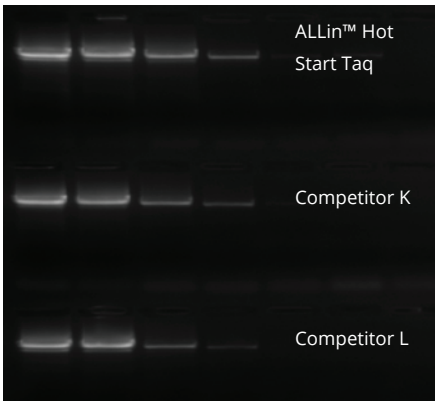
BENEFITS

- Small molecular inhibition hot-start technology combined with advanced buffer - advantages over classical hot- start Taq
- Outperforming sensitivity & specificity - low copy target detection
- Higher yields under standard and fast cycling
- Increased success in amplification of longer templates (6 kb)
- Robust amplification of GC rich templates
- 5X ALLin™ PCR Buffer contains optimal Mg^{2+} and dNTPs

PRODUCT DETAILS

highQu ALLin™ Hot Start Taq Polymerase is the superior sensitive hot-start enzyme. The activity at room temperature is blocked by small molecular inhibitor. Enzyme becomes active only after heating what allows for highly specific and extremely sensitive amplification, no primer dimer formation and no background.
In combination with the optimized ALLin™ buffer enzyme provides higher success rates in demanding PCR applications like amplification of complex or longer templates and fast cycling. ALLin™ Hot Start Taq DNA Polymerase has the same PCR accuracy like Taq DNA Polymerase, and produces A-tailed products suitable for ligating into TA cloning vectors. For the maximum convenience use 2X ALLin™ Hot Start Taq Mastermixes. *See next pages.*

PERFORMANCE



highQu ALLin™ Hot Start Taq DNA Polymerase shows better yields and higher sensitivity compared to competitors.

PCR of a 0.4 kb fragment, from human genomic DNA, under fast cycling conditions. Starting template 100 ng with further 10 fold dilutions.

PROTOCOL

- Take typical measures to prevent PCR cross over contamination, keep your bench clean, wear gloves, use sterile tubes and filter pipet tips.
- Include a no-template control and positive control in parallel.
- Thaw and keep reagents on ice. Mix well before use.
- The longer the amplicon, the longer the extension time: Use 15 sec/kb extension.
- Use 90 sec extension for multiplexing. Do not use fast cycling for multiplexing.
- Run an annealing temperature gradient from 55°C to 65°C to choose the best specificity conditions.

IN VITRO RESEARCH USE ONLY

✓ Prepare a 50 µl reaction:

Rev. & For. Primers	0.1-0.4 µM final each (≤2 µl of 10 µM)
cDNA Template or gDNA Template	< 100 ng or 5-500 ng
5X ALLin™ PCR Buffer	10 µl
Water (PCR Water, WAT0110)	to 49 µl
ALLin™ Hot Start Taq DNA Polymerase, 5 u/µl	0.25 - 1 µl

✓ Mix gently, avoid bubbles.
✓ Place into the instrument set like:

Initial denaturation	1 cycle: 95°C – 1-2 min
Denaturation	40 cycles: 95°C - 15 sec
Annealing	40 cycles: 55-65°C – 15 sec
Extension	40 cycles: 72°C – 1-90 sec (15 sec/kb)

✓ Store probes for short time on ice, for long at -20°C.

ALLin™ HS Red Taq Mastermix, 2X

bulk quantities available

CAT.#	SIZE	COMPONENTS	COMPONENT COMPOSITION
HSM0301	200 r of 50 µl	5 x 1 ml - ALLin™ HS Red Taq Mastermix, 2X 5 x 1 ml - PCR Water	1X mastermix contains 0.25 mM dNTPs, 3 mM $MgCl_2$, enhancers, stabilizers, red electrophoresis tracking dye and density reagents for gel loading.
HSM0305	1000 r of 50 µl	25 x 1 ml - ALLin™ HS Red Taq Mastermix, 2X 25 x 1 ml - PCR Water	
Storage:	*In the dark at -20°C.*		

APPLICATIONS

- Hot-start PCR up to 6 kb for a direct gel loading
- Crude sample and colony PCR
- Low copy target detection
- Amplification of complex (GC/AT rich) templates
- Fast PCR
- TA cloning
- Multiplex hot-start PCR

BENEFITS

- Outperforming sensitivity & specificity - low copy number target detection and no background
- Higher yields under standard and fast cycling
- Increased sensitivity and success in amplification of longer templates (6 kb), robust amplification of GC rich templates
- Premixed with the red dye and density reagents for direct loading on the gels after the PCR

PRODUCT DETAILS

highQu ALLin™ Hot Start Taq DNA Polymerase is the superior sensitive enzyme. The activity at room temperature is blocked by small molecular inhibitor. Enzyme becomes active only after heating what allows for highly specific and extremely sensitive amplification, no primer dimer formation and no background.
In combination with the optimized ALLin™ buffer enzyme provides higher success rates in demanding PCR applications like amplification of complex or longer templates and fast cycling.
ALLin™ Hot Start Taq DNA Polymerase has the same PCR accuracy like Taq DNA Polymerase, and produces A-tailed products suitable for ligating into TA cloning vectors.

The convenience of ALLin™ Hot Start Taq DNA Polymerase is maximized by the use of 2X Mastermixes providing the additional advantage of reduced pipetting and minimized errors.

ALLin™ HS Red Taq Mastermix, 2X is premixed with the red dye and density reagents for direct loading on the gels after the PCR. In a 2% agarose TAE gel the dye migrates with~350 bp DNA, in 1% agarose TAE gel with ~ 600 bp DNA fragments.

The mastermix is even supplied with PCR water.

ALLin™ HS Red Taq Mastermix, 2X is also a key component in highQu SampleIN™ Direct PCR Kit (DPK0101/5), ensuring outstanding PCR results with crude samples.

PROTOCOL

- Take typical measures to prevent PCR cross over contamination, keep your bench clean, wear gloves, use sterile tubes and filter pipet tips.
- Include a no-template control and positive control in parallel.
- Thaw and keep reagents on ice. Mix well before use.
- The longer the amplicon, the longer the extension time: Use 15 sec/kb extension.
- Use 90 sec extension for multiplexing. Do not use fast cycling for multiplexing.
- Run an annealing temperature gradient from 55°C to 65°C to choose the best specificity conditions.
- ALLin™ HS Red Taq Mastermix, 2X is premixed with red dye and density reagents for direct loading on the gels. In a 2% agarose TAE gel the dye migrates with~350 bp DNA, in 1% agarose TAE gel with ~ 600 bp DNA fragments.

IN VITRO RESEARCH USE ONLY

✓ Prepare a 50 µl reaction:

Rev. & For. Primers	0.1-0.4 µM final each (≤2 µl of 10 µM)
cDNA Template or gDNA Template	< 100 ng or 5-500 ng
PCR Water	to 25 µl
ALLin™ HS Red Taq Mastermix, 2X	25 µl

✓ Mix gently, avoid bubbles.
✓ Place into the instrument set like:

Initial denaturation	1 cycle: 95°C – 1-2 min
Denaturation	40 cycles: 95°C - 15 sec
Annealing	40 cycles: 55-65°C – 15 sec
Extension	40 cycles: 72°C – 1-90 sec (15 sec/kb)

✓ Load probes on the agarose gel. The red loading dye is included in the mastermix.
✓ Store probes for short time on ice, for long at -20°C.

ALLin™ Hot Start Taq Mastermix, 2X

bulk quantities available

CAT.#	SIZE	COMPONENTS	COMPONENT COMPOSITION
HSM0201	200 r of 50 µl	5 x 1 ml - ALLin™ Hot Start Taq Mastermix, 2X 5 x 1 ml - PCR Water	1X mastermix contains 0.25 mM dNTPs, 3 mM $MgCl_2$, enhancers, stabilizers.
HSM0205	1000 r of 50 µl	25 x 1 ml - ALLin™ Hot Start Taq Mastermix, 2X 25 x 1 ml - PCR Water	
Storage:	*In the dark at -20°C.*		

APPLICATIONS

- Sensitive hot-start PCR up to 6 kb
- Low copy target detection
- Amplification of complex (GC/AT rich) templates
- Fast PCR
- TA cloning
- Multiplex hot-start PCR
- Colony PCR

BENEFITS

- Small molecular inhibition hot-start technology combined with advanced buffer - advantages over classical hot- start Taq
- Outperforming sensitivity & specificity - low copy number target detection and no background
- Higher yields under standard and fast cycling
- Increased sensitivity and success in amplification of longer templates (6 kb)
- Robust amplification under difficult conditions - GC rich templates

PRODUCT DETAILS

highQu ALLin™ Hot Start Taq DNA Polymerase is the superior sensitive enzyme. The activity at room temperature is blocked by small molecular inhibitor. Enzyme becomes active only after heating what allows for highly specific and extremely sensitive amplification, no primer dimer formation and no background.
In combination with the optimized ALLin™ buffer enzyme provides higher success rates in demanding PCR applications like amplification of complex or longer templates and fast cycling.
ALLin™ Hot Start Taq DNA Polymerase has the same PCR accuracy like Taq DNA Polymerase, and produces A-tailed products suitable for ligating into TA cloning vectors.

The convenience of ALLin™ Hot Start Taq DNA Polymerase is maximized by the use of 2X Mastermix providing the additional advantage of reduced pipetting and minimized errors.

The mastermix is even supplied with PCR water.

PROTOCOL

- Take typical measures to prevent PCR cross over contamination, keep your bench clean, wear gloves, use sterile tubes and filter pipet tips.
- Include a no-template control and positive control in parallel.
- Thaw and keep reagents on ice. Mix well before use.
- The longer the amplicon, the longer the extension time: Use 15 sec/kb extension.
- Use 90 sec extension for multiplexing. Do not use fast cycling for multiplexing.
- Run an annealing temperature gradient from 55°C to 65°C to choose the best specificity conditions.

✓ Prepare a 50 µl reaction:

Rev. & For. Primers	0.1-0.4 µM final each (≤2 µl of 10 µM)
cDNA Template or gDNA Template	< 100 ng or 5-500 ng
PCR Water	to 25 µl
ALLin™ Hot Start Taq Mastermix, 2X	25 µl

✓ Mix gently, avoid bubbles.
✓ Place into the instrument set like:

Initial denaturation	1 cycle: 95°C – 1-2 min
Denaturation	40 cycles: 95°C - 15 sec
Annealing	40 cycles: 55-65°C – 15 sec
Extension	40 cycles: 72°C – 1-90 sec (15 sec/kb)

✓ Store probes for short time on ice, for long at -20°C.

IN VITRO RESEARCH USE ONLY

SampleIN™ Direct PCR Kit

bulk quantities available

CAT.#	SIZE	COMPONENTS	COMPONENT COMPOSITION
DPK0101	80 r of 50 µl	1.6 ml - DPK Lysis Buffer, 5X 0.8 ml - DPK Protease Buffer, 10X 2 x 1 ml - ALLin™ HS Red Taq Mastermix, 2X	DPK Lysis Buffer, 5X contains all components required for an efficient lysis of mammalian tissue samples. DPK Protease Buffer, 10X contains proteases to eliminate sample proteins. 1X ALLin™ HS Red Taq Mastermix contains hot-start enzyme, 0.25 mM dNTPs, 3 mM $MgCl_2$, enhancers, stabilizers, red electrophoresis tracking dye and density reagents for gel loading
DPK0105	400 r of 50 µl	5 x 1.6 ml - DPK Lysis Buffer, 5X 5 x 0.8 ml - DPK Protease Buffer, 10X 10 x 1 ml - ALLin™ HS Red Taq Mastermix, 2X	

Storage: *In the dark at -20°C.* ALLin™ HS Red Taq Mastermix can be ordered separately: HSM0301 / HSM0305.

APPLICATIONS

- Fast direct PCR without template purification
- Mouse genotyping and knockout analysis
- Direct PCR from mouse tail or ear, mammalian tissues (including FFPE), hair follicle, buccal swabs and blood (including EDTA or FTA samples), plants

BENEFITS

- Ready-to load PCR in 50 minutes without template purification
- Single-tube 15 min DNA extraction combined with fast hot-start PCR
- Red dye in the PCR master mix for direct gel loading
- High yields under standard or fast cycling conditions
- Success with GC/AT rich templates

PRODUCT DETAILS

SampleIN™ Direct PCR Kit is a premium tool for a fast direct PCR eliminating the need of tedious template purification. The kit is excellent for direct PCR from mouse tail or ear, mammalian tissues, hair follicle, buccal swabs, blood, and plant material. Rapid 15 min DNA extraction using DPK Lysis and Protease Buffers in a single tube generates PCR template extract which is further amplified under fast cycling conditions with a hot-start Taq master mix that includes red dye for direct gel loading. In a 2% agarose TAE gel the red dye migrates with~350 bp DNA, in 1% agarose TAE gel with ~ 600 bp DNA fragments. The ALLin™ HS Red Taq Mastermix includes a hot start Taq DNA Polymerase what ensures high yield, specific, low background amplification. Mix components allow for a fast PCR cycling and increase success when working with complex templates or multiplexing. Generated A-tailed PCR products are suitable for ligating into TA cloning vectors, sequencing and other applications.

SAMPLE GUIDELINES

Sample (fresh or frozen)	Amount	Extraction vol.
Mouse tail	2 mm or 3-5 mg	100 µl
Mouse ear	2 mm^2 or 3-5 mg	100 µl
Mammalian tissue	5 mg	100 µl
FFPE Tissue	2 mm^2 of 10 µm section	100 µl
Blood (fresh/EDTA)	2 µl	100 µl
Blood Guthrie cards	2 mm^2	100 µl
Blood FTA/FTA Elute cards	2 mm^2	100 µl
Hair follicle	2 follicles	100 µl
Buccal swab	1 swab	300 µl

Sample amounts can be slightly increased for better yields, but too much material may cause inefficient lysis and PCR inhibition.

SampleIN™ Direct PCR Kit (upper) gives higher PCR yields from different dilutions of mouse tail sample extracts compared to competitors' kits (lower).

I. SAMPLE DNA EXTRACTION PROTOCOL

- Take typical measures to prevent contamination, keep your bench clean, wear gloves, and use sterile tubes.
- Thaw DPK Buffers at room temperature. Mix well before use.
- Prepare a 100 µl extraction reaction in a sterile vial (use 3x larger volumes of all reagents for buccal swab):

Sample amount	as above in SAMPLE GUIDELINES
DPK Lysis Buffer, 5X	20 µl
DPK Protease Buffer, 10X	10 µl
PCR Water (not supplied)	70 µl
✓ Mix gently, avoid bubbles. Place into the instrument set like:	
Lysis	75°C - 5 min. Vortex twice during lysis.
Protease inactivation	95°C - 10 min

- Add 900 µl of PCR Water. Centrifuge 1 min to pellet cell debris.
- Remove supernatant into the sterile tube.
- Store it at -20°C for several months or use immediately for PCR.

IN VITRO RESEARCH USE ONLY

II. PCR PROTOCOL

✓ Prepare a 50 µl PCR reaction:

Rev. & For. Primers	0.1-0.4 µM final each (≤ 2 µl of 10 µM)
Template	1-5 µl of extraction supernatant
PCR Water	to 25 µl
ALLin™ HS Red Taq Mastermix, 2X	25 µl
✓ Mix gently, avoid bubbles. Place into the instrument set like:	
Initial denaturation	1 cycle: 95°C - 2 min
Denaturation	40 cycles: 95°C - 15 sec
Annealing	40 cycles: 55-65°C – 15 sec
Extension	40 cycles: 72°C – 15 sec/kb (90 sec for multiplex)

✓ Load probes on the gel. The red loading dye is included in PCR mix.

✓ Store probes for short time on ice, for long at -20°C.

The use of this product in certain countries for certain applications may be covered by patents and may require a license

ALLin™ RPH Polymerase

CAT.#	SIZE	COMPONENTS	COMPONENT COMPOSITION
HLE0101	250 u	250 u - ALLin™ RPH Polymerase, 5 u/µl 2 x 1 ml - 5X ALLin™ RPH Buffer	Enzyme in storage buffer. 1X ALLin™ RPH Buffer contains 0.25 mM dNTPs, 3 mM $MgCl_2$, enhancers, stabilizers.
HLE0105	1250 u	5 x 250 u - ALLin™ RPH Polymerase, 5 u/µl 10 x 1 ml - 5X ALLin™ RPH Buffer	
Storage:	*In the dark at -20°C.*		

APPLICATIONS

- Amplification of difficult & complex (GC/AT rich) templates
- Long PCR (up to 35 kb) with higher fidelity
- Colony & crude sample PCR
- Multiplex PCR
- TA cloning

BENEFITS

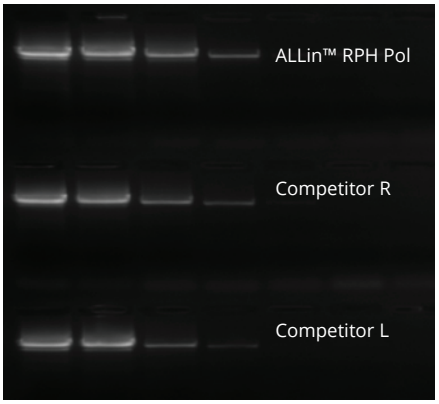
- RPH – Robust, Proofreading, Hot-start Polymerase
- Low-copy number target detection ensured by small molecular inhibitor hot-start
- Long PCR (up to 35 kb), higher-fidelity (5X higher than Taq) – ensured by proofreading activity
- High yields under standard and fast cycling
- Robust - GC or AT rich templates, crude sample PCR
- 5X ALLin™ PCR Buffer contains optimal Mg^{2+} and dNTPs

PRODUCT DETAILS

highQu ALLin™ RPH Polymerase (Robust, Proofreading, Hot-start Polymerase) is the versatile engineered enzyme combining best polymerase properties for excellence in most demanding PCR applications, like low copy detection, long or high fidelity PCR, amplification of complex templates, crude sample PCR and multiplexing.

ALLin™ RPH Polymerase has 5 times higher fidelity than Taq DNA Polymerase and produces A-tailed products suitable for ligating into TA cloning vectors.
For the maximum convenience the 2X ALLin™ RPH Mastermix is available. *See next page.*

PERFORMANCE



ALLin™ RPH Polymerase ensures higest sensitivity amplification of 25 kb target from lowest amounts of human genomic DNA. The starting template concentration is 200 ng of human genomic DNA with 2x further dilutions. 25kb fragment of the p53 gene was amplified

PROTOCOL

- Take typical measures to prevent PCR cross over contamination, keep your bench clean, wear gloves, use sterile tubes and filter pipet tips.
- Include a no-template control and positive control in parallel.
- Thaw and keep reagents on ice. Mix well before use.
- The longer the amplicon, the longer the extension time: Use 15 sec/kb extension for amplicons of < 5 kb.
- Use 40 - 60 sec/kb extension for amplicons of 5 - 35 kb.
- Use 90 sec extension for multiplexing. Do not use fast cycling for multiplexing.
- Run an annealing temperature gradient from 55°C to 65°C to choose the best specificity conditions.

IN VITRO RESEARCH USE ONLY

✓ Prepare a 50 µl reaction:

Rev. & For. Primers	0.1-0.4 µM final each (≤2 µl of 10 µM)
cDNA Template or gDNA Template	< 100 ng or 5-500 ng
5X ALLin™ RPH Buffer	10 µl
Water (PCR Water, WAT0110)	to 49 µl
ALLin™ RPH Polymerase, 5 u/µl	0.25 - 1 µl

✓ Mix gently, avoid bubbles.
✓ Place into the instrument set like:

Initial denaturation	1 cycle: 95°C – 1 min
Denaturation	25-35 cycles: 95°C - 15 sec
Annealing	25-35 cycles: 55-65°C – 15 sec
Extension	25-35 cycles: 72°C – ~10 min (10 kb)

✓ Store probes for short time on ice, for long at -20°C.

ALLin™ RPH Mastermix, 2X

bulk quantities available

CAT.#	SIZE	COMPONENTS	COMPONENT COMPOSITION
HLM0101	200 r of 50 µl	5 x 1 ml - ALLin™ RPH Mastermix, 2X 5 x 1 ml - PCR Water	1X mastermix contains 0.25 mM dNTPs, 3 mM $MgCl_2$, enhancers, stabilizers.
HLM0105	1000 r of 50 µl	25 x 1 ml - ALLin™ RPH Mastermix, 2X 25 x 1 ml - PCR Water	
Storage:	*In the dark at -20°C.*		

APPLICATIONS

- Amplification of difficult & complex (GC/AT rich) templates
- Long PCR (up to 35 kb) with higher fidelity
- Colony & crude sample PCR
- Multiplex PCR
- TA cloning

BENEFITS

- RPH – Robust, Proofreading, Hot-start Polymerase
- Low-copy number target detection ensured by small molecular inhibitor hot-start
- Long PCR (up to 35 kb), higher-fidelity (5X higher than Taq) – ensured by proofreading activity
- High yields under standard and fast cycling
- Robust - GC or AT rich templates, crude sample PCR

PRODUCT DETAILS

highQu ALLin™ RPH Polymerase (Robust, Proofreading, Hot-start Polymerase) is the versatile engineered enzyme combining best polymerase properties for excellence in most demanding PCR applications, like low copy detection, long or high fidelity PCR, amplification of complex templates, crude sample PCR and multiplexing.
ALLin™ RPH Polymerase has 5 times higher fidelity than Taq DNA Polymerase and produces A-tailed products suitable for ligating into TA cloning vectors.

The convenience of ALLin™ RPH Polymerase is maximized by the use of 2X Mastermix providing the additional advantage of reduced pipetting and minimized errors.

The mastermix is even supplied with PCR water.

PROTOCOL

- Take typical measures to prevent PCR cross over contamination, keep your bench clean, wear gloves, use sterile tubes and filter pipet tips.
- Include a no-template control and positive control in parallel.
- Thaw and keep reagents on ice. Mix well before use.
- The longer the amplicon, the longer the extension time: Use 15 sec/kb extension for amplicons of < 5 kb.
- Use 40-60 sec/kb extension for amplicons of 5-35 kb.
- Use 90 sec extension for multiplexing. Do not use fast cycling for multiplexing.
- Run an annealing temperature gradient from 55°C to 65°C to choose the best specificity conditions.

IN VITRO RESEARCH USE ONLY

✓ Prepare a 50 µl reaction:

Rev. & For. Primers	0.1-0.4 µM final each (≤2 µl of 10 µM)
cDNA Template or gDNA Template	< 100 ng or 5-500 ng
PCR Water	to 25 µl
ALLin™ RPH Mastermix, 2X	25 µl

✓ Mix gently, avoid bubbles.
✓ Place into the instrument set like:

Initial denaturation	1 cycle: 95°C – 1-2 min
Denaturation	25-40 cycles: 95°C - 15 sec
Annealing	25-40 cycles: 55-65°C – 15 sec
Extension	25-40 cycles: 72°C – ~10 min (example for 10 kb)

✓ Store probes for short time on ice, for long at -20°C.

ALLin™ HiFi DNA Polymerase

bulk quantities available

CAT.#	SIZE	COMPONENTS	COMPONENT COMPOSITION
HLE0201	200 u	200 u - ALLin™ HiFi DNA Polymerase, 2 u/µl 3 x 1 ml - 5X ALLin™ HiFi Buffer	Enzyme in storage buffer. 1X ALLin™ HiFi Buffer contains 0.25 mM dNTPs, 3 mM $MgCl_2$, enhancers, stabilizers.
HLE0205	1000 u	5 x 200 u - ALLin™ HiFi DNA Polymerase, 2 u/µl 15 x 1 ml - 5X ALLin™ HiFi Buffer	
Storage:	*In the dark at -20°C.*		

APPLICATIONS

- High-fidelity PCR up to 10 kb
- Long PCR up to 10 kb
- Amplification of complex (GC/AT rich) templates
- Fast high-fidelity PCR
- Blunt cloning
- Crude sample & colony PCR with high fidelity

BENEFITS

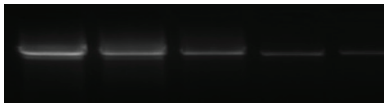
- Engineered proofreading enzyme and advanced buffer
- 50 x higher fidelity than classic Taq
- Increased sensitivity, high yield under standard and fast cycling
- Increased success in PCR of longer templates (10 kb)
- Robust on GC/AT rich templates, crude samples
- 5X ALLin™ PCR Buffer contains optimal Mg^{2+} and dNTPs

PRODUCT DETAILS

highQu ALLin™ HiFi DNA Polymerase is the outperforming high-fidelity enzyme derived from Pfu polymerase by introducing several point mutations. The robust engineered enzyme in combination with the optimized ALLin™ buffer provides higher fidelity (50X higher than Taq), better performance, increased success in demanding applications like amplification of complex or longer templates, crude sample PCR and fast cycling.

ALLin™ HiFi DNA Polymerase produces blunt-ended products suitable for ligating into blunt vectors.

PERFORMANCE



highQu ALLin™ HiFi DNA Polymerase (above) shows better yields and higher sensitivity compared to P enzyme from competitor N (below).
PCR of a 1 kb fragment of 60% GC GAPDH, from human genomic DNA.
The template is diluted 2 fold over 8 orders of magnitude, starting from 100 ng.

PROTOCOL

- Take typical measures to prevent PCR cross over contamination, keep your bench clean, wear gloves, use sterile tubes and filter pipet tips.
- Include a no-template control and positive control in parallel.
- Thaw and keep reagents on ice. Mix well before use.
- The longer the amplicon, the longer the extension time: 30 sec/kb.
- Run an annealing temperature gradient from 55°C to 65°C to choose the best specificity conditions.

IN VITRO RESEARCH USE ONLY

✓ Prepare a 50 µl reaction:

Rev. & For. Primers	0.1-0.4 µM final each (≤2 µl of 10 µM)
cDNA Template or gDNA Template	< 100 ng or 5-500 ng
5X ALLin™ HiFi Buffer	10 µl
Water (PCR Water, WAT0110)	to 49 µl
ALLin™ HiFi DNA Polymerase, 2 u/µl	0.5 µl

✓ Mix gently, avoid bubbles.
✓ Place into the instrument set like:

Initial denaturation	1 cycle: 95°C - 1 min
Denaturation	25-35 cycles: 95°C - 15 sec
Annealing	25-35 cycles: 55-65°C – 15 sec
Extension	25-35 cycles: 72°C – 30 sec (30 sec/kb)

✓ Store probes for short time on ice, for long at -20°C.

ALLin™ Mega HiFi DNA Polymerase

CAT.#	SIZE	COMPONENTS	COMPONENT COMPOSITION
HLE0301	100 u	100 u - ALLin™ Mega HiFi DNA Polymerase, 2 u/µl 1.5 ml - 5X ALLin™ Mega HiFi Buffer	Enzyme in storage buffer. 1X ALLin™ Mega HiFi Buffer contains 1 mM dNTP, 3 mM MgCl2, enhancers, stabilizers.
HLE0305	500 u	5x 100 u - ALLin™ Mega HiFi DNA Polymerase, 2 u/µl 5x 1.5 ml - 5X ALLin™ Mega HiFi Buffer	
Storage:	*In the dark at -20°C.*		

APPLICATIONS

- Fast high-fidelity PCR (up to 100 x Taq)
- Long PCR up to 20 kb
- Amplification of complex (GC/AT rich) templates
- Sequencing, including NGS
- Blunt-end cloning and other applications

BENEFITS

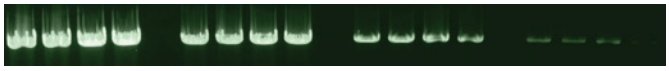
- Fast, high yield PCR with the fidelity 100x higher than Taq
- Up to 20 kb long PCR even from complex templates
- Increased processivity for faster amplification and higher yield
- High thermostability for better denaturation of GC rich templates
- Best choice for NGS and other sequencing applications
- 5X ALLin™ Buffer includes optimal Mg2+ and dNTP amount

PRODUCT DETAILS

Derived from our HiFi polymerase, the highQu ALLin™ Mega HiFi DNA Polymerase provides much lower error rate PCR with a 100 higher fidelity compared to Taq. The ALLin™ Mega HiFi DNA Polymerase is engineered to be much faster and to generate higher yield of long PCR products up to 20 kb from complex GC-rich templates. Therefore the ALLin™ Mega HiFi DNA Polymerase is an excellent choice for longer and very complex PCR applications where the highest fidelity is demanded.
It is an enzyme of choice for cloning and all kind of sequencing applications including NGS.
Generated blunt-ended PCR products are suitable for ligation into blunt vectors.
For the maximum convenience the 2X ALLin™Mega HiFi Red Mastermix (HLM0301) and ALLin™Mega HiFi Mastermix (HLM0201) are available.

PERFORMANCE

Applying fast cycling conditions and high denaturation temperatures, ALLin™ Mega HiFi DNA Polymerase provides consistent long PCR results independently from template amount



20 ng, 99-95°C den. *5 ng, 99-95°C den.* *1 ng, 99-95°C den.* *0,2 ng, 99-95°C den.*

Amplification of ~6 kb fragment from mouse gDNA using ALLin™ Mega HiFi DNA Polymerase at different template concentrations from 20 ng down to 0,2 ng, at different denaturation temperatures for each concentration from 99°C down to 98, 97, 95 °C. Fast cycling protocol -10 seconds per kilobase: Denaturation 1 min at 95°C, 30 cycles: 10 sec 95-99°C; 10 sec 67°C; 60 sec 72°C, 2 min 72°C.

PROTOCOL

- Take typical measures to prevent PCR cross over contamination, keep your bench clean, wear gloves, use sterile tubes and filter pipet tips.
- Include a no-template control and positive control in parallel.
- Thaw and keep reagents on ice. Mix very well before use. Mixing of the buffer is very important for the final yield!
- For complex, GC rich templates, use 99-100°C denaturation temperature, it might help to increase the yield.
- For established PCRs, try two-step cycling protocol with a combined annealing-denaturation step of 70°C (68°C to 75°C).
- Run an annealing temperature gradient (2°C increments) from 60°C to 66°C to choose the best conditions.
- The longer the amplicon, the longer the extension time: depending on the complexity of the template, perform extension from 10 sec/kb to 30 sec/kb. Longer extension for more complex templates is needed.

IN VITRO RESEARCH USE ONLY

✓ Prepare a 50 µl reaction:

Rev. & For. Primers	0.2-0.6 µM final each (≤2 µl of 10 µM)
cDNA Template or gDNA Template	< 100 ng or 10-200 ng
5X ALLin™ Mega HiFi Buffer	10 µl
Water (PCR Water WAT0110)	to 49.5 µl
ALLin™ Mega HiFi DNA Polymerase, 2 u/µl	0.5 µl

✓ Mix gently, avoid bubbles.
✓ Place into the instrument set like:

Initial denaturation	1 cycle: 95°C – 1 min
Denaturation	25-35 cycles: 95°C - 15 sec
Annealing	25-35 cycles: 60-66°C – 15 sec
Extension	25-35 cycles: 72°C – 30 sec. (10-30 sec/kb)

✓ Store probes for short time on ice, for long at -20°C.

ALLin™ Mega HiFi Red Mastermix, 2X

bulk quantities available

CAT.#	SIZE	COMPONENTS	COMPONENT COMPOSITION
HLM0301	100 r of 50 µl	2 x 1.25 ml - ALLin™ Mega HiFi Red Mastermix, 2X 3 x 1 ml – PCR Water	1X mastermix contains 1 mM dNTPs, 3 mM MgCl2, enhancers, stabilizers, red electrophoresis tracking dye and density reagents for gel loading.
HLM0305	500 r of 50 µl	10 x 1.25 ml - ALLin™ Mega HiFi Red Mastermix, 2X 13 x 1 ml – PCR Water	
Storage:	*In the dark at -20°C.*		

APPLICATIONS

- Fast high-fidelity PCR (up to 100 x Taq)
- Direct gel loading option
- Long PCR up to 20 kb
- Amplification of complex (GC/AT rich) templates
- Sequencing, including NGS
- Blunt-end cloning and other applications

BENEFITS

- Fast, high yield PCR with the fidelity 100x higher than Taq
- Up to 20 kb long PCR even from complex templates
- Increased processivity for faster amplification and higher yield
- High thermostability for better denaturation of GC rich templates
- Best choice for NGS and other sequencing applications
- Red master mix for direct gel loading, supplied with water

PRODUCT DETAILS

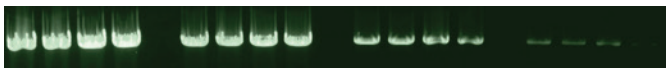
Derived from our HiFi polymerase, the highQu ALLin™ Mega HiFi DNA Polymerase provides much lower error rate PCR with a 100 higher fidelity compared to Taq. The ALLin™ Mega HiFi DNA Polymerase is engineered to be much faster and to generate higher yield of long PCR products up to 20 kb from complex GC-rich templates. Therefore the ALLin™ Mega HiFi DNA Polymerase is an excellent choice for longer and very complex PCR applications where the highest fidelity is demanded. It is an enzyme of choice for cloning and all kind of sequencing applications including NGS.

Generated blunt-ended PCR products are suitable for ligation into blunt vectors.

The convenience of ALLin™ Mega HiFi DNA Polymerase is maximized by the use of 2X Red Mastermix providing the advantage of reduced pipetting and direct gel loading.

PERFORMANCE

Applying fast cycling conditions and high denaturation temperatures, ALLin™ Mega HiFi DNA Polymerase provides consistent long PCR results independently from template amount



20 ng, 99-95°C den. *5 ng, 99-95°C den.* *1 ng, 99-95°C den.* *0,2 ng, 99-95°C den.*

Amplification of ~6 kb fragment from mouse gDNA using ALLin™ Mega HiFi DNA Polymerase at different template concentrations from 20 ng down to 0,2 ng, at different denaturation temperatures for each concentration from 99°C down to 98, 97, 95 °C. Fast cycling protocol -10 seconds per kilobase: Denaturation 1 min at 95°C, 30 cycles: 10 sec 95-99°C; 10 sec 67°C; 60 sec 72°C, 2 min 72°C.

PROTOCOL

- Take typical measures to prevent PCR cross over contamination, keep your bench clean, wear gloves, use sterile tubes and filter pipet tips.
- Include a no-template control and positive control in parallel.
- Thaw and keep reagents on ice. Mix very well before use. Mixing of the mix is very important for the final yield!
- For complex, GC rich templates, use 99-100°C denaturation temperature, it might help to increase the yield.
- For established PCRs, try two-step cycling protocol with a combined annealing-denaturation step of 70°C (68°C-75°C).
- Run an annealing temperature gradient (2°C increments) from 60°C to 66°C to choose the best conditions.
- The longer the amplicon, the longer the extension time: depending on the complexity of the template, perform extension from 10 sec/kb to 30 sec/kb. Longer extension for more complex templates is needed.

IN VITRO RESEARCH USE ONLY

✓ Prepare a 50 µl reaction:

Rev. & For. Primers	0.2-0.6 µM final each (≤2 µl of 10 µM)
cDNA Template or gDNA Template	< 100 ng or 10-200 ng
ALLin™ Mega HiFi Red Mastermix, 2X	25 µl
PCR Water (WAT0110)	to 50 µl

✓ Mix gently, avoid bubbles.
✓ Place into the instrument set like:

Initial denaturation	1 cycle: 95°C - 1 min
Denaturation	25-35 cycles: 95°C - 15 sec
Annealing	25-35 cycles: 60-66°C – 15 sec
Extension	25-35 cycles: 72°C– 30 sec (10-30 sec/kb)

✓ Store probes for short time on ice, for long at -20°C.
✓ Load probes directly on the agarose gel. ALLin™ Mega HiFi Red Mastermix is premixed with dye and density reagents for direct loading. In a 2% agarose TAE gel the dye migrates with~350 bp DNA, in 1% agarose - with ~ 600 bp DNA.

ALLin™ Mega HiFi Mastermix, 2X

bulk quantities available

CAT.#	SIZE	COMPONENTS	COMPONENT COMPOSITION
HLM0201	100 r of 50 µl	2 x 1.25 ml - ALLin™ Mega HiFi Mastermix, 2X 3 x 1 ml - PCR Water	1X mastermix contains 1 mM dNTPs, 3 mM $MgCl_2$, enhancers, stabilizers.
HLM0205	500 r of 50 µl	10 x 1.25 ml - ALLin™ Mega HiFi Mastermix, 2X 13 x 1 ml - PCR Water	

Storage: *In the dark at -20°C.*

APPLICATIONS

- Fast high-fidelity PCR (up to 100 x Taq)
- Long PCR up to 20 kb
- Amplification of complex (GC/AT rich) templates
- Sequencing, including NGS
- Blunt-end cloning and other applications

BENEFITS

- Fast, high yield PCR with the fidelity 100x higher than Taq
- Up to 20 kb long PCR even from complex templates
- Increased processivity for faster amplification and higher yield
- High thermostability for better denaturation of GC rich templates
- Best choice for NGS and other sequencing applications
- Mix format for maximum convenience, supplied with water

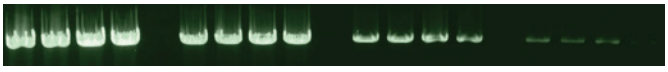
PRODUCT DETAILS

Derived from our HiFi polymerase, the highQu ALLin™ Mega HiFi DNA Polymerase provides much lower error rate PCR with a 100 higher fidelity compared to Taq. The ALLin™ Mega HiFi DNA Polymerase is engineered to be much faster and to generate higher yield of long PCR products up to 20 kb from complex GC-rich templates. Therefore the ALLin™ Mega HiFi DNA Polymerase is an excellent choice for longer and very complex PCR applications where the highest fidelity is demanded. It is an enzyme of choice for cloning and all kind of sequencing applications including NGS. Generated blunt-ended PCR products are suitable for ligation into blunt vectors.

The convenience of ALLin™ Mega HiFi DNA Polymerase is maximized by the use of 2X Mastermix providing the additional advantage of reduced pipetting and minimized errors.

PERFORMANCE

Applying fast cycling conditions and high denaturation temperatures, ALLin™ Mega HiFi DNA Polymerase provides consistent long PCR results independently from template amount



20 ng, 99-95°C den. *5 ng, 99-95°C den.* *1 ng, 99-95°C den.* *0,2 ng, 99-95°C den.*

Amplification of ~6 kb fragment from mouse gDNA using ALLin™ Mega HiFi DNA Polymerase at different template concentrations from 20 ng down to 0,2 ng, at different denaturation temperatures for each concentration from 99°C down to 98, 97, 95 °C. Fast cycling protocol -10 seconds per kilobase: Denaturation 1 min at 95°C, 30 cycles: 10 sec 95-99°C; 10 sec 67°C; 60 sec 72°C, 2 min 72°C.

PROTOCOL

- Take typical measures to prevent PCR cross over contamination, keep your bench clean, wear gloves, use sterile tubes and filter pipet tips.
- Include a no-template control and positive control in parallel.
- Thaw and keep reagents on ice. Mix very well before use. Mixing of the mix is very important for the final yield!
- For complex, GC rich templates, use 99-100°C denaturation temperature, it might help to increase the yield.
- For established PCRs, try two-step cycling protocol with a combined annealing-denaturation step of 70°C (68°C to 75°C).
- Run an annealing temperature gradient (2°C increments) from 60°C to 66°C to choose the best conditions.
- The longer the amplicon, the longer the extension time: depending on the complexity of the template, perform extension from 10 sec/kb to 30 sec/kb. Longer extension for more complex templates is needed.

✓ Prepare a 50 µl reaction:

Rev. & For. Primers	to 0.2 - 0.6 µM each (~2µl of 10 µM)
cDNA Template or gDNA Template	< 100 ng or 10 - 200 ng
ALLin™ Mega HiFi Mastermix, 2X	25 µl
PCR Water (WAT0110)	to 50 µl

✓ Mix gently, avoid bubbles.
✓ Place into the instrument set like:

Initial denaturation	1 cycle: 95°C – 1min
Denaturation	25-35 cycles: 95°C - 15 sec
Annealing	25-35 cycles: 60-66°C – 15 sec
Extension	25-35 cycles: 72°C – 30 sec (10-30 sec/kb)

✓ Store probes for short time on ice, for long at -20°C.

IN VITRO RESEARCH USE ONLY

ALLin™ Mega HS HiFi DNA Polymerase

CAT.#	SIZE	COMPONENTS	COMPONENT COMPOSITION
HLE0401	100 u	100 u - ALLin™ Mega HS HiFi DNA Polymerase, 2 u/µl 1.5 ml - 5X ALLin™ Mega HS HiFi Buffer	Enzyme in storage buffer. 1X ALLin™ Mega HS HiFi Buffer contains 1 mM dNTP, 3 mM $MgCl_2$, enhancers, stabilizers.
HLE0405	500 u	5 x 100 u - ALLin™ Mega HS HiFi DNA Polymerase, 2 u/µl 5 x 1.5 ml - 5X ALLin™ Mega HS HiFi Buffer	

Storage: *In the dark at -20°C.*

APPLICATIONS

- Sequencing, including NGS library preparation
- Hot start PCR, multiplexing
- Fast high-fidelity PCR (up to 100 x Taq)
- Long PCR up to 20 kb
- Amplification of complex (GC/AT rich) templates
- Blunt-end cloning and other applications

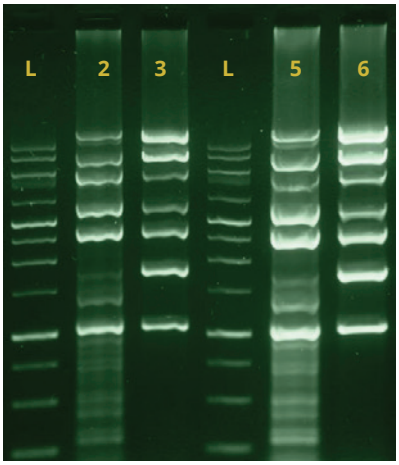
BENEFITS

- Hot start for increased sensitivity and great multiplexing results
- Fast, high yield PCR with the fidelity 100x higher than Taq
- Up to 20 kb long PCR even from complex templates
- Increased processivity for faster amplification and higher yield
- High thermostability for better denaturation of GC rich templates
- Best choice for NGS library prep. and other sequencing applications
- 5X ALLin™ Buffer includes optimal Mg2+ and dNTP amount

PRODUCT DETAILS

Derived from our HiFi polymerase, the highQu ALLin™ Mega HS HiFi DNA Polymerase provides much lower error rate PCR with a 100 higher fidelity compared to Taq. Compared to Mega HiFi, this hot start enzyme version allows for even higher sensitivity and specificity of PCR as well as for a room temperature reaction setup, and is excellent choice for multiplex reactions.
The ALLin™ Mega HS HiFi DNA Polymerase is engineered to be much faster and to generate higher yield of long PCR products up to 20 kb from complex GC-rich templates. Therefore the ALLin™ Mega HS HiFi DNA Polymerase is an excellent choice for longer and very complex PCR applications where the highest fidelity is demanded. It is an enzyme of choice for cloning and all kind of sequencing applications including NGS.
Generated blunt-ended PCR products are suitable for ligation into blunt vectors.

PERFORMANCE



High sensitivity multiplex PCR results achieved with Allin™ Mega HS HiFi DNA Polymerase

Gel analysis of multiplex PCR reactions - compared to competitor enzyme (2; 5), the Allin™ Mega HS HiFi DNA Polymerase (3; 6) gives more specific multiplex result.

PROTOCOL

- Take typical measures to prevent PCR cross over contamination, keep your bench clean, wear gloves, use sterile tubes and filter pipet tips.
- Include no-template and positive control in parallel.
- Thaw and keep reagents on ice. Mix very well before use. Mixing of the buffer is very important for the final yield!
- For complex, GC rich templates, use 99-100°C denaturation temperature, it might help to increase the yield.
- For established PCRs, try 2-step cycling with a combined annealing-denaturation step of 70°C (68°C to 75°C).
- Run an annealing temperature gradient (2°C increments) from 60°C to 66°C to choose the best conditions.
- The longer the amplicon, the longer the extension time: depending on the complexity of the template, perform extension from 10 to 30 sec/kb. Longer extension for more complex templates is needed. For multiplexing, start with extension time needed for the longest fragment.

IN VITRO RESEARCH USE ONLY

✓ Prepare a 50 µl reaction:

Rev. & For. Primers	To 0.2 - 0.6 µM each (~2µl of 10 µM)
cDNA Template or gDNA Template	< 100 ng or 10-200 ng
5X ALLin™ Mega HS HiFi Buffer	10 µl
Water (PCR Water, WAT0110)	to 49.5 µl
ALLin™ Mega HS HiFi DNA Polymerase, 2 u/µl	0.5 µl

✓ Mix gently, avoid bubbles.
✓ Place into the instrument set like:

Initial denaturation	1 cycle: 95°C - 1 min
Denaturation	25-35 cycles: 95°C - 15 sec
Annealing	25-35 cycles: 60-66°C – 15 sec
Extension	25-35 cycles: 72°C – 30 sec (30 sec/kb)

✓ Store probes for short time on ice, for long at -20°C.

ALLin™ Mega HS HiFi Red Mastermix, 2x

bulk quantities available

CAT.#	SIZE	COMPONENTS	COMPONENT COMPOSITION
HLM0501	100 r of 50 µl	2 x 1.25 ml - ALLin™ Mega HS HiFi Red Mastermix, 2X 3 x 1 ml - PCR Water	1X mastermix contains 1 mM dNTPs, 3 mM MgCl2, enhancers, stabilizers, red electrophoresis tracking dye and density reagents for gel loading.
HLM0505	500 r of 50 µl	10 x 1.25 ml - ALLin™ Mega HS HiFi Red Mastermix, 2X 13 x 1 ml - PCR Water	

Storage: *In the dark at -20°C.*

APPLICATIONS

- Sequencing, including NGS library preparation
- Hot start PCR, multiplexing
- Direct gel loading option
- Fast high-fidelity PCR (up to 100 x Taq)
- Long PCR up to 20 kb
- Amplification of complex (GC/AT rich) templates
- Blunt-end cloning and other applications

BENEFITS

- Hot start for increased sensitivity and great multiplex results
- Fast, high yield PCR with the fidelity 100x higher than Taq
- Up to 20 kb long PCR even from complex templates
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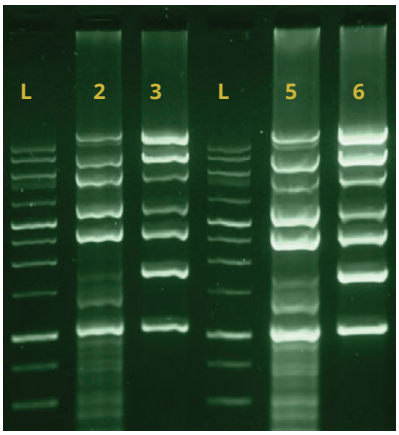
PRODUCT DETAILS

Derived from our HiFi polymerase, the highQu ALLin™ Mega HS HiFi DNA Polymerase provides much lower error rate PCR with a 100 higher fidelity compared to Taq. Compared to Mega HiFi, this hot start enzyme version allows for even higher sensitivity and specificity of PCR as well as for a room temperature reaction setup, and is excellent choice for multiplex reactions. The ALLin™ Mega HS HiFi DNA Polymerase is engineered to be much faster and to generate higher yield of long PCR products up to 20 kb from complex GC-rich templates. Therefore the ALLin™ Mega HS HiFi DNA Polymerase is an excellent choice for longer and very complex PCR applications where the highest fidelity is demanded. It is an enzyme of choice for cloning and all kind of sequencing applications including NGS.
Generated blunt-ended PCR products are suitable for ligation into blunt vectors.

PERFORMANCE



High sensitivity multiplex PCR results achieved with Allin™ Mega HS HiFi DNA Polymerase

Gel analysis of multiplex PCR reactions - compared to competitor enzyme (2; 5), the Allin™ Mega HS HiFi DNA Polymerase (3; 6) gives more specific multiplex result.

The convenience of ALLin™ Mega HS HiFi DNA Polymerase is maximized by the use of 2X Red Mastermix providing the advantage of reduced pipetting and direct gel loading. ALLin™ Mega HS HiFi Red Mastermix, 2X is premixed with red dye and density reagents for direct loading on the gels after the PCR.

PROTOCOL

- Take typical measures to prevent PCR cross over contamination, keep your bench clean, wear gloves, use sterile tubes and filter pipet tips.
- Include a no-template control and positive control in parallel.
- Thaw and keep reagents on ice. Mix very well before use. Mixing of the mix is very important for the final yield!
- For complex, GC rich templates, use 99-100°C denaturation temperature, it might help to increase the yield.
- For established PCRs, try two-step cycling protocol with a combined annealing-denaturation step of 70°C (68°C to 75°C).
- Run an annealing temperature gradient (2°C increments) from 60°C to 66°C to choose the best conditions.
- The longer the amplicon, the longer the extension time: depending on the complexity of the template, perform extension from 10 sec/kb to 30 sec/kb. Longer extension for more complex templates is needed. For multiplexing, start with extension time needed for the longest fragment.

IN VITRO RESEARCH USE ONLY

✓ Prepare a 50 µl reaction:

Rev. & For. Primers	To 0.2 - 0.6 µM each (~2µl of 10 µM)
cDNA Template or gDNA Template	< 100 ng or 10-200 ng
ALLin™ Mega HS HiFi Red Mastermix, 2X	25 µl
PCR Water	to 50 µl

✓ Mix gently, avoid bubbles.
✓ Place into the instrument set like:

Initial denaturation	1 cycle: 95°C – 1-2 min
Denaturation	25-35 cycles: 95°C - 15 sec
Annealing	25-35 cycles: 60-66°C – 15 sec
Extension	25-35 cycles: 72°C – 30 sec (10-30 sec/kb)

✓ Store probes for short time on ice, for long at -20°C.
✓ Load probes directly on the agarose gel. ALLin™ Mega HiFi Red Mastermix, 2X is premixed with red dye and density reagents for direct loading after the PCR. In a 2% agarose TAE gel the dye migrates with~350 bp DNA, in 1% agarose - with ~ 600 bp DNA.

ALLin™ Mega HS HiFi Mastermix, 2X

bulk quantities available

CAT.#	SIZE	COMPONENTS	COMPONENT COMPOSITION
HLM0401	100 r of 50 µl	2 x 1.25 ml - ALLin™ Mega HS HiFi Mastermix, 2X 3 x 1 ml - PCR Water	1X mastermix contains 1 mM dNTPs, 3 mM MgCl2, enhancers, stabilizers
HLM0405	500 r of 50 µl	10 x 1.25 ml - ALLin™ Mega HS HiFi Mastermix, 2X 13 x 1 ml – PCR Water	
Storage:	*In the dark at -20°C.*		

APPLICATIONS

- Sequencing, including NGS library preparation
- Hot start PCR, multiplexing
- Fast high-fidelity PCR (up to 100 x Taq)
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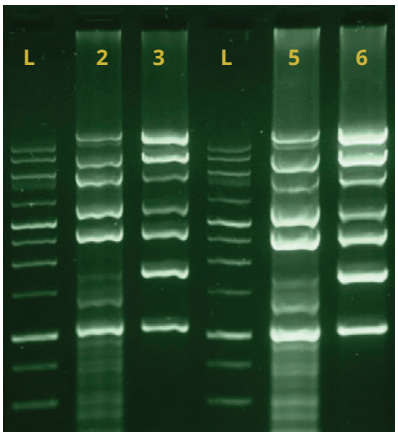
Generated blunt-ended PCR products are suitable for ligation into blunt vectors.

BENEFITS

- Hot start for increased sensitivity and great multiplex results
- Fast, high yield PCR with the fidelity 100x higher than Taq
- Up to 20 kb long PCR even from complex templates
- Increased processivity for faster amplification and higher yield
- High thermostability for better denaturation of GC rich templates
- Best for NGS library prep. and other sequencing applications
- Master mix format for maximum convenience, supplied with water

PERFORMANCE



High sensitivity multiplex PCR results achieved with Allin™ Mega HS HiFi DNA Polymerase

Gel analysis of multiplex PCR reactions - compared to competitor enzyme (2; 5), the Allin™ Mega HS HiFi DNA Polymerase (3; 6) gives more specific multiplex result.

The convenience of ALLin™ Mega HS HiFi DNA Polymerase is maximized by the use of 2X Mastermix providing the advantage of reduced pipetting steps.

PROTOCOL

- Take typical measures to prevent PCR cross over contamination, keep your bench clean, wear gloves, use sterile tubes and filter pipet tips.
- Include a no-template control and positive control in parallel.
- Thaw and keep reagents on ice. Mix very well before use. Mixing of the mix is very important for the final yield!
- For complex, GC rich templates, use 99-100°C denaturation temperature, it might help to increase the yield.
- For established PCRs, try two-step cycling protocol with a combined annealing-denaturation step of 70°C (68°C to 75°C).
- Run an annealing temperature gradient (2°C increments) from 60°C to 66°C to choose the best conditions.
- The longer the amplicon, the longer the extension time: depending on the complexity of the template, perform extension from 10 sec/kb to 30 sec/kb. Longer extension for more complex templates is needed. For multiplexing, start with extension time needed for the longest fragment.

IN VITRO RESEARCH USE ONLY

✓ Prepare a 50 µl reaction:

Rev. & For. Primers	To 0.2 - 0.6 µM each (~2µl of 10 µM)
cDNA Template or gDNA Template	< 100 ng or 10-200 ng
ALLin™ Mega HS HiFi Mastermix, 2X	25 µl
PCR Water	to 50 µl

✓ Mix gently, avoid bubbles.
✓ Place into the instrument set like:

Initial denaturation	1 cycle: 95°C - 1 min
Denaturation	25-35 cycles: 95°C - 15 sec
Annealing	25-35 cycles: 60-66°C – 15 sec
Extension	25-35 cycles: 72°C – 30 sec (10-30 sec/kb)

✓ Store probes for short time on ice, for long at -20°C.

highQu product range for reverse transcription and RT PCR provides convenient kits and master mixes to ensure safety of RNA samples and to shorten time to results due to the reduced number of pipeting steps and due to accelerated reactions.
Our thermostable reverse transcriptase allows for high yields of full lengths transcripts of up to 12-15 kb and for cDNA synthesis from complex templates.

Want to try one? Order a sample today at www.highqu.com/Samples
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RT PCR and Reverse Transcription Enzymes and Kits

RT PCR & Reverse Transcription Selection

	1Step RT PCR Kit	HighScriber™ Reverse Transcriptase Mix	qScriber™ cDNA Synthesis Kit	SecurRIN™ Advanced RNase Inhibitor
	page 59	*page 60*	*page 61*	*page 62*
Short description	One-step RT PCR in one tube	cDNA synthesis of long transcripts at high temperature	Unbiased cDNA synthesis for qPCR	Prevents RNA degradation
Enhanced cDNA synthesis	●	●	●	
GC rich and complex templates	●	●	●	
Full-length cDNA transcripts up to 15 kb		●		
High sensitivity	●	●	●	
One-step RT-PCR	●			
Two-step RT-PCR		● ●	●	
Two-step RT-qPCR		●	● ●	
RNA protection from RNases	●	●	●	●

can be used in all applications to inhibit RNAses to prevent RNA degradation

1Step RT PCR Kit

CAT.#	SIZE	COMPONENTS	COMPONENT COMPOSITION
RTK0201	100 r of 50 µl	2 x 1.25 ml - 1Step RT PCR Mastermix, 2X 2 x 0.125 ml - RT2 Mix, 20X 3 x 1 ml - PCR Water	1X mastermix contains hot-start Taq DNA Polymerase, 0.25 mM dNTPs, 3 mM $MgCl_2$, enhancers, stabilizers. RT2 Mix is a 20X concentrated blend of reverse transcriptase and RNase inhibitor

Storage: *In the dark at -20°C.*

APPLICATIONS

- One step RT-PCR
- RT-PCR of complex GC/AT rich templates
- Fast RT-PCR
- TA cloning

BENEFITS

- Easy to use combination of the RT mix with the RT-PCR mastermix - reverse transcription and PCR in one tube
- RT Mix contains RNase inhibitor and thermostable reverse transcriptase (up to 55°C) allowing for high cDNA yields
- RT PCR Mastermix allows for sensitive low copy number targets detection due to proprietary hot-start
- High yields under standard and fast cycling conditions and on GC/AT rich templates

PRODUCT DETAILS

highQu 1Step RT PCR Kit combines the blend of Reverse Transcriptase and RNase Inhibitor for efficient reverse transcription and the PCR Mastermix for subsequent amplification of cDNA in the same tube. RT2 Mix, 20X is a blend of the engineered MMuLV (stable at 40-55°C allowing for high yields of long transcripts) with an efficient Ribonuclease Inhibitor protecting the template RNA from RNases. The resulting cDNA is then amplified by the 1Step RT PCR Mastermix, 2X. The PCR mastermix contains our proprietary Hot Start Taq DNA Polymerase. The activity at room temperature is blocked by small molecular inhibitor. Enzyme becomes active only after heating what allows for highly specific and extremely sensitive amplification, no primer dimer formation and no background.

In combination with the optimized buffer the enzyme provides higher success rates in demanding PCR applications like amplification of complex or longer templates and fast cycling. Hot Start Taq DNA Polymerase has the same PCR accuracy like Taq DNA Polymerase and produces A-tailed products suitable for ligating into TA cloning vectors.

For the maximum convenience the Kit includes even the PCR Water to set up the reaction, so the only thing you need to take care is the high quality RNA template.

PROTOCOL

- RNA is extremely sensitive to degradation by RNases present everywhere. Take care to protect RNA from degradation and to prevent PCR cross over contamination, keep your bench clean, wear gloves, use sterile tubes and filter pipet tips.
- Include a no-template control, no RT2 Mix control and positive control in parallel.
- Thaw and keep reagents on ice. Mix well before use.
- Perform cDNA synthesis 10 min at 45°C, 20 min for>1 kb, increase temperature to 55°C for complex templates.
- The longer the amplicon, the longer the extension time: Use 15 sec/kb extension for amplicons of <3 kb.
- Use 40-60 sec/kb extension for amplicons of 5-10 kb.
- Run an annealing temperature gradient from 58°C to 65°C to choose the best specificity conditions.

IN VITRO RESEARCH USE ONLY

✓ Prepare a 50 µl reaction:

Rev. & For. Primers	0.2-0.4 µM final (≤2 µl of 10 µM each)
Total RNA mRNA	1 pg to 1 µg or > 0.01 pg
PCR Water	to 22.5 µl
1Step RT PCR Mastermix, 2X	25 µl
RT2 Mix, 20X	2.5 µl

✓ Mix gently, avoid bubbles.
✓ Place into the instrument set like:

Reverse transcription	1 cycle: 45 - 55°C – 10 to 20 min
Initial denaturation	1 cycle: 95°C – 2 min
Denaturation	40 cycles: 95°C - 10 sec
Annealing	40 cycles: 60-65°C – 10 sec
Extension	40 cycles: 72°C – 30-60 sec (15 sec/kb)

✓ Store probes for short time on ice, for long at -20°C.

HighScriber™ Reverse Transcriptase Mix, 20X

bulk quantities available

CAT.#	SIZE	COMPONENTS	COMPONENT COMPOSITION
RTM0301	10000 u / 50 r	2 x 25 µl - HighScriber™ Reverse Transcriptase Mix, 20X 2x 0.2 ml - 5X ALLin™ HighScriber Reaction Buffer	Enzyme Mix contains HighScriber™ RT at 200 u/µl concentration, Ribonuclease Inhibitor and glycerol. Reaction Buffer includes magnesium, DTT and dNTPs.
RTM0305	50000 u / 250 r	10 x 25 µl - HighScriber™ Reverse Transcriptase Mix, 20X 10 x 0.2 ml - 5X ALLin™ HighScriber Reaction Buffer	

Storage: *In the dark at -20°C.*

APPLICATIONS

- cDNA synthesis of up to 15 kb long transcripts
- Template generation for RT-PCR & RT-qPCR
- cDNA synthesis from complex templates

BENEFITS

- Thermostable Reverse Transcriptase blended with RNase Inhibitor for an efficient cDNA synthesis
- High yields of full lengths transcripts up to 12-15 kb
- cDNA synthesis from complex templates at up to 55°C
- High sensitivity detection from 1 pg of total RNA template

PRODUCT DETAILS

The HighScriber™ Reverse Transcriptase Mix is a premium tool for the high efficiency reverse transcription of up to 12-15 kb long cDNA. Mix includes HighScriber™ RT at 200 u/µl concentration and Ribonuclease Inhibitor for safe cDNA synthesis. HighScriber™ Reverse Transcriptase allows for high detection sensitivity from 1 pg of total RNA. The wide reaction temperature range (38°C - 55°C) ensures efficient cDNA synthesis from complex or GC rich templates. The enzyme uses ssRNA or ssDNA as a template, possesses no detectable Ribonuclease H activity specific to RNA in RNA-DNA hybrids. A highly reduced Ribonuclease H activity allows for transcription of full lengths long transcripts.
HighScriber™ RT can be used for RACE as it has terminal transferase activity - adds cytosines to 3' ends of cDNA.

SPECIFICATIONS

- Optimal activity at 45-50°C
- Temperature range 38-55°C
- Inactivation at 85°C for 10 min

The Ribonuclease inhibitor premixed with the RT ensures RNA protection from ribonuclease degradation. Supplied 5X ALLin™ HighScriber Buffer includes everything you need for the cDNA synthesis reaction: it contains $MgCl_2$, dNTPs, enhancers, stabilizers.The only things to add is the template RNA and primer.

One unit is defined as the amount of enzyme that will incorporate 1 nmol of dTTP into acid-insoluble material in a total reaction volume of 50 µl in 10 minutes at 37°C using poly (rA) oligo $(dT)_{18}$ as template.

PROTOCOL

- RNA is extremely sensitive to degradation by RNases present everywhere. Take care to protect RNA from degradation keep your bench clean, wear gloves, use sterile tubes and filter pipet tips.
- Check the integrity of RNA prior to cDNA synthesis in denaturing agarose gel.
- Include positive control in parallel.
- Thaw and keep reagents on ice. Mix well before use.
- For best results, optimize the template and primer amount.
- Do not exceed the recommended amount of the enzyme.
- Perform reaction for 30-50 min, for short transcripts 15-30 min are sufficient.
- Choose optimal reaction temperature in a range of 42-55°C.

  Do not add Ribonuclease Inhibitor and dNTPs, as they are already included in supplied Mix and reaction buffer.

✓ Prepare a 20 µl reaction:

5X ALLin™ HighScriber Reaction Buffer	4 µl (includes dNTPs!)
Oligo dT primer or Random primer or Specific primer	0.5 µg or 0.2 µg or 15-20 pmol
Total RNA or Poly-A mRNA	1 pg to 5 µg or 1 pg to 0.5 µg
Water (PCR Water, WAT0110)	to 19 µl

✓ Mix gently, avoid bubbles.
✓ Heat 5 min at 65°C, spin, place on ice for 1 min.
✓ Incubate 2 min at 42°C for Oligo dT and for Specific primer or 10 min at 25°C for Random primer to anneal.
✓ Add 1 µl HighScriber™ RTase Mix, 20X and mix well.
✓ Incubate 30-50 min at 50°C to synthesize cDNA.
✓ Inactivate at 85°C for 10 min.
✓ Store reactions at -20°C or on ice for an immediate use.
✓ Use 2-5 µl of this reaction mix per 50 µl PCR reaction.
✓ Use 1-2 µl of this reaction mix per 20 µl qPCR reaction.

IN VITRO RESEARCH USE ONLY

qScriber™ cDNA Synthesis Kit

CAT.#	SIZE	COMPONENTS	COMPONENT COMPOSITION
RTK0101	25 r of 20 µl	25 µl - qScriber™ Enzyme Blend, 20X 100 µl - 5X qScriber™ Reaction Mix 1 ml – PCR Water	Enzyme Blend Storage buffer contains Tris, 50% glycerol and other components. 5X qScriber™ Reaction Mix contains dNTPs, $MgCl_2$, anchored oligo(dT), random hexamers and other components.
RTK0104	100 r of 20 µl	4 x 25 µl - qScriber™ Enzyme Blend, 20X 4 x 100 µl - 5X qScriber™ Reaction Mix 2 x 1 ml – PCR Water	

Storage: *In the dark at -20°C.*

APPLICATIONS

- cDNA template generation for qPCR or PCR
- Unbiased, efficient cDNA synthesis
- Detection of low target amounts
- cDNA synthesis from complex templates

BENEFITS

- Thermostable HighScriber™Reverse Transcriptase blended with Ribonuclease Inhibitor for efficient cDNA synthesis
- Optimized reaction mix with oligo (dT) and random primers for unbiased representation of mRNA ends
- cDNA synthesis from complex templates at up to 55°C
- High sensitivity detection from 1 pg of total RNA template

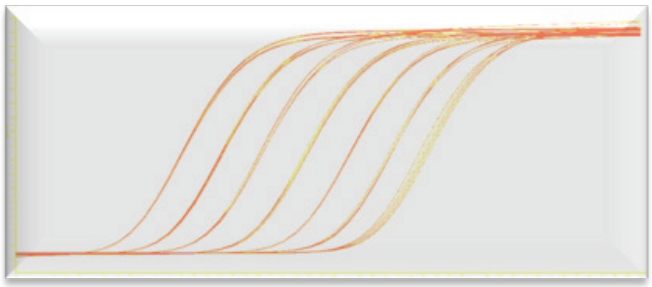
PRODUCT DETAILS

The qScriber™ cDNA Synthesis Kit is a highly efficient and simple-to-use system for cDNA synthesis eliminating the need for tedious reaction optimization. The qScriber™ Enzyme Blend ensures high sensitivity detection from low copy number targets. The highly active and thermostable HighScriber™ Reverse Transcriptase blended with RNase Inhibitor allows for an efficient cDNA synthesis and reaction safety. The wide reaction temperature range (38°C - 55°C) ensures efficient transcription from GC rich templates.

The 5X qScriber™ Reaction Mix includes optimal concentrations of magnesium and dNTPs and a combination of anchored oligo (dT) and random hexamers for unbiased representation of mRNA ends. The kit is an optimal choice for generating high quality cDNA from viral RNA, miRNA or other targets for qPCR or for PCR.

PERFORMANCE

qScriber™ cDNA Synthesis Kit provides excellent results within the very broad range of the total RNA amount used.



4 pg, 40 pg, 400 pg, 4 ng, 40 ng, 400 ng and 4 µg of mouse total RNA were used for cDNA synthesis under the standard qScriber™ cDNA Synthesis Kit protocol conditions. An aliquot from each reaction was taken for subsequent qPCR with ORA™ qPCR Green Mix to amplify a 70 bp fragment of the mouse RN18S gene. All reactions independently from the initial amount of RNA were 100% efficient.

PROTOCOL

- RNA is sensitive to degradation by RNases present everywhere. Take care to protect RNA from degradation keep your bench clean, wear gloves, use sterile tubes and filter pipet tips.
- Include positive and negative controls in parallel.
- Thaw and keep reagents on ice. Mix well before use.
- Do not add any other components into the reaction with exception of the template and the supplied reagents.
- The recommended reaction temperature is 42-50°C. For GC rich templates, the temperature can be increased up to 55°C.

IN VITRO RESEARCH USE ONLY

✓ Prepare a 20 µl reaction:

5X qScriber™ Reaction Mix	4 µl
qScriber™ Enzyme Blend, 20X	1 µl
Total RNA	1 pg to 5 µg
PCR Water	up to 20 µl

✓ Mix gently, avoid bubbles.
✓ Incubate 30 min at 42-50°C to synthesize cDNA.
✓ Inactivate the enzyme at 85°C for 10 min.

✓ Store reactions at -20°C or on ice for an immediate use.
✓ Use 2-4 µl of this reaction mix per 20 µl qPCR reaction.

SecurRIN™ Advanced RNase Inhibitor

bulk quantities available

CAT.#	SIZE	COMPONENTS	COMPONENT COMPOSITION
RNI0301	2500 u	1 x 65 µl - SecurRIN™ Advanced RNase Inhibitor, 40 u/µl	Storage buffer contains 50% glycerol, 8mM DTT and other components.
RNI0305	12500 u	5 x 65 µl - SecurRIN™ Advanced RNase Inhibitor, 40 u/µl	
Storage:	*At -20°C. The enzyme is very stable, the performance remains the same even after a few weeks storage at +37°C.*		

APPLICATIONS

- Prevention of RNA degradation during:
  - ✓ cDNA synthesis
  - ✓ RNA extraction and storage
  - ✓ in vitro transcription and translation
- Monoclonal antibody preparation procedures

BENEFITS

- Efficient RNAse inhibition at higher temperatures up to 60°C
- RNA protection from hydrolysis by RNases A, B and C
- Economical & stable for weeks at ambient temperature
- Robust, works well at 5.5 to 9.0 pH, with 0.5 - 1 mM DTT

PRODUCT DETAILS

SecurRIN™ Advanced RNase Inhibitor is a premium tool for RNA protection from degradation during enzymatic reactions, storage or extraction. It is an extraordinary stable and robust enzyme: it works at up to 60°C temperature, remains active after weeks of room temperature exposure and multiple freezing thawing cycles. It is active in different buffer conditions within a broad pH range of 5.5 to 9.0, and within 0.5 - 1 mM concentration of DTT. SecurRIN™ Advanced RNase Inhibitor is a 50 kDa non-covalent inhibitor of RNase A, RNase B, and RNase C binding them in a 1 to 1 ratio. It is a recombinant protein derived from E. coli strain carrying human placenta RNase Inhibitor gene.
SecurRIN™ Advanced RNase Inhibitor does not inhibit RNAses H, 1, T1, T2 and S1 Nuclease. It influences neither the activity nor the performance of DNA polymerases and of Reverse Transcriptases. The enzyme is free from DNAses and RNAses.

SPECIFICATIONS

- Active in all common buffers used for RNA work
- Acceptable pH range is 5.5 to 9.0
- Stable at ambient high temperature of up to 60°C
- Optimally performs at 0.5 - 1 mM DTT concentration
- 1 - 2 units of the RNase Inhibitor are typically enough for each 1 µl of RNA reaction. Optimal enzyme amount depends on RNAse contamination grade
- 1µl of the enzyme is recommended for a standard cDNA synthesis reaction of 20 µl volume
- Binds and inhibits RNAse A, RNAse B and RNAse C at 1:1 ratio
- Does not inhibit RNAse H, RNAse 1, RNAse T1, RNAse T2

One unit is required to inhibit 5 ng of RNAse A by 50% (measuring the hydrolysis of cytidine 2', 3'-cyclic monophosphate).

PROTOCOL example (please follow recommendations for Reverse Transcriptase you use)

- RNA is extremely sensitive to degradation by RNases present everywhere. Take care to protect RNA from degradation keep your bench clean, wear gloves, use sterile tubes and pipet tips.
- Check the integrity of RNA prior to cDNA synthesis in denaturing agarose gel.
- Add SecurRIN™ Advanced RNase Inhibitor in to the cDNA synthesis reaction (~40 units for 20 µl reaction tube) as the first component.
- In case high contamination with RNAses is suspected, use 2 µl of the RNase Inhibitor. However, to avoid too much DTT in PCR, then use less of cDNA reaction mix as template for further PCR.
- Include positive/negative controls in parallel for each reaction.
- Thaw and keep reagents on ice. Mix well before use.
- For best results, optimize the template and primer amount.
- Choose optimal reaction temperature in a range recommended for your Reverse Transcriptase.

Perform reaction as recommended for your Reverse Transcriptase. Given temperatures and times are just approximate suggestions.

✓ Prepare a 20 µl reaction:

SecurRIN™ Advanced RNase Inhibitor, 40 u/µl	1 µl (40 u)
10 mM dNTP Mix (NUM0201)	2 µl (1 mM final)
Total RNA or Poly-A mRNA	1 pg to 5 µg or 1 pg to 0.5 µg
Oligo dT primer or Random primer or Specific primer	0.5 µg or 0.2 µg or 15-20 pmol
Water (PCR Water, WAT0110)	to 15 µl

- ✓ Mix gently, avoid bubbles.
- ✓ Heat 5 min at 65°C, spin, place on ice for 1 min.
- ✓ Add the 4 µl of 5X RT Reaction Buffer.
- ✓ Add 1 µl of Reverse Transcriptase, 200 u/µl and mix well.
- ✓ Incubate 2 min at 42°C for Oligo dT and for Specific primer or 10 min at 25°C for Random primer to anneal.
- ✓ Incubate 30-50 min at 50°C to synthesize cDNA.
- ✓ Inactivate at 85°C for 10 min.
- ✓ Store reactions at -20°C or on ice for an immediate use.
- ✓ Use 2-5 µl of this reaction mix per 50 µl PCR reaction.
- ✓ Use 1-2 µl of this reaction mix per 20 µl qPCR reaction.

IN VITRO RESEARCH USE ONLY

NA Isolation and PCR-related Reagents and Kits

Proteinase K MBG Solution

bulk quantities available

CAT.#	SIZE	COMPONENTS	COMPONENT COMPOSITION
PRK0101	1 ml / 20 mg	1 ml - Proteinase K MBG Solution, 20 mg/ml	Storage buffer contains glycerol, Tris-HCl and Calcium Acetate.
PRK0105	5 ml / 100 mg	5 x 1 ml - Proteinase K MBG Solution, 20 mg/ml	

Storage: *At -20°C . The product is very stable. It can also be stored at +4°C and shipped at ambient temperature.*

APPLICATIONS

- Protein hydrolysis
- DNAse and RNase decontamination
- Protein degradation during the procedures of:
  - ✓ Tissue/Cell lysis
  - ✓ DNA extraction
  - ✓ RNA extraction

BENEFITS

- Recombinant broad spectrum, non-specific protease
- Ready-to-use solution with a very high specific activity
- Economical and stable for weeks at ambient temperature
- Robust, works well at a wide variety of reaction conditions: up to 56°C and in a presence of urea, SDS and guanidinium salts
- Safe – DNase and RNase free to ensure integrity of isolated nucleic acids

PRODUCT DETAILS

Proteinase K MBG (Molecular Biology Grade) Solution is a serine peptidase with a very high specific activity and a broad spectrum of protein digestion possibilities.

The solution is designed to be used for protein degradation (up to tetrapeptides) during the cell lysis and RNA/DNA extraction procedures under harsh reaction conditions - such as at higher temperatures and in the presence of detergents. The enzyme efficiently degrades DNases and RNases during the nucleic acid isolation process. The high purity of the Proteinase K and controlled absence of both DNAse and RNase contamination ensures the integrity of nucleic acids.

100% enzyme activity (when stored at -20°C) is guaranteed for at least two years after production. However, our experiments demonstrated that the proteinase remains close to 90% active even when stored at 37°C for 18 months.

SPECIFICATIONS

The enzyme is supplied as a 20 mg/ml concentrated solution with an average specific activity of more than 800 u/ml.

- Active in all common buffers used for cell lysis and RNA/DNA extraction, in a presence of urea, SDS and guanidinium salts
- Stable at high temperatures of up to 56°C
- Can be inactivated by heating at 65oC for 20 minutes or at 75°C for 10 minutes
- Active in a pH range of 4–12 with an optimum pH 7.5–8.0
- The Proteinase K gene is from T. album and is expressed in a yeast host.
- The quality limit of allowed host DNA presence is ≤ 0.25 pg/u as measured by qPCR.

Unit Definition

Folin & Ciocalteu's method - One unit is required to hydrolyze urea-denatured hemoglobin producing color equivalent of 1 µmol tyrosine in 1 minute at 37°C and pH 7.5, 1 unit = 1 m Anson unit.

PROTOCOL RECOMMENDATIONS

- The 20 mg/ml Proteinase K MBG Solution is ready to use.
- Mix the solution well before each use.
- The recommended working concentration of the Proteinase K is in the range of 0.05 to 1 mg/ml in the final reaction mixture.
- Proteinase K activity is stimulated in the presence of >1% SDS and 1-4 M urea.
- The activity of the enzyme is not inhibited by metal chelators or trypsin and chymotrypsin inhibitors.
- Calcium ions present in the storage buffer increase the thermal stability of the enzyme and protect it against autolysis.
- Store, open and use Proteinase K in separate facilities from where other enzymes and proteins are used to protect them from accidental degradation. Discard gloves and pipet tips immediately, and clean surfaces after handling Proteinase K.

RESEARCH USE ONLY

Synthetic Carrier RNA

bulk quantities available

CAT.#	SIZE	COMPONENTS	COMPONENT COMPOSITION
SCR0101	1 ml	1 ml – Synthetic Carrier RNA, 1 mg/ml	Aqueous solution containing synthetic RNA (polyinosinic acid) at 1 mg/ml concentration.
SCR0105	5 ml	5 ml – Synthetic Carrier RNA, 1 mg/ml	
SCR0201	1 ml	1 ml – Synthetic Carrier RNA, 10 mg/ml	Aqueous solution containing synthetic RNA (polyinosinic acid) at 10 mg/ml concentration.
SCR0205	5 ml	5 ml – Synthetic Carrier RNA, 10 mg/ml	
Storage:	*At -20°C. Can be stored at ambient temperature up to 2 weeks. Can be shipped at ambient temperature. High concentration RNA solution may be difficult to pipet. To reduce the viscosity, warm it in 37°C thermostat for a few minutes and mix well.*		

APPLICATIONS

- All molecular biology applications where an increased yield of RNA or DNA is required, such as:
  - ✓ RNA extraction/isolation procedures
  - ✓ DNA extraction/isolation procedures
  - ✓ Clean-up and reprecipitation of RNA or DNA

BENEFITS

- Animal and yeast-free aqueous solution of synthetic RNA
- Inert coprecipitating agent helping to increase the concentration of target nucleic acids in low-concentration solutions
- Shows no inhibition in RT-PCR, PCR and qPCR reactions
- Stable - can be stored and shipped at ambient temperature

PRODUCT DETAILS

highQu Synthetic Carrier RNA is designed to be used in a wide range of nucleic acid purification and precipitation procedures as a carrier and co-precipitant of nucleic acids. It is especially useful to increase the RNA or DNA yield in low concentration solutions; such as in viral RNA extraction from human specimen samples.
In contrast to commonly used carrier RNAs - such as tRNA, yeast RNA, or sonicated salmon sperm DNA - our Synthetic Carrier RNA is free from contaminating animal or yeast RNA. Coprecipitated RNA and DNA can be directly used for all common downstream applications, such as PCR or RT-PCR, as well as the highly sensitive qPCR.

NOTES

The use of carrier RNAs for coprecipitation of nucleic acids may interfere with spectrophotometric measurements.

The presence of carrier RNAs in RNA or DNA solutions may have some influence on certain reactions performed by enzymes that act on all nucleic acid molecules – such as T4 Polynucleotide Kinase or Terminal DNA Transferase.

PROTOCOL RECOMMENDATIONS

- Use Synthetic Carrier RNA in DNA or RNA solutions during alcohol precipitation step.
- To maximize the yield of nucleic acids, add Synthetic Carrier RNA and mix it well with DNA/RNA sample before adding salt (sodium acetate) and ethanol or isopropanol.
- Use the following amounts of Synthetic Carrier RNA:
  - ✓ Recommended final concentration in precipitation solution is 10–20 µg/ml
  - ✓ For example, add 1 µl of 10 mg/ml Synthetic Carrier RNA to 200 µl of RNA or DNA sample which will be precipitated with 3 x volumes of ethanol.
  - ✓ Alternatively, add 5 µl of 1 mg/ml Synthetic Carrier RNA into 100 µl of RNA or DNA sample which will be precipitated with 3 x volumes of ethanol.

RESEARCH USE ONLY

PCRbeam™ Fast PCR Detection Kit

CAT.#	SIZE	COMPONENTS	COMPONENT COMPOSITION
PDK0101	50 tests	50 - PCRbeam™ Membrane Strip 10 ml - PCRbeam™ Detection Buffer	PCRbeam™ Membrane Strips are coated with biotin-ligand (for test band) and anti-FITC antibody in gold conjugate PCRbeam™ Detection Buffer is Tris-buffered saline.

Storage: *Store the Buffer in the dark at +4°C. Membrane strips shall be stored at ambient temperature, protected from humidity.*

APPLICATIONS

- Low throughput PCR, LAMP, RPA based tests
- Sensitive detection of specific amplification products
- Fast and 20x more sensitive alternative to EtBr stained gels
- Economical alternative to qPCR-based detection

BENEFITS

- Sensitive detection of PCR, LAMP, RPA gene-specific products
- No gel loading after PCR, no ethidium bromide handling
- Saved costs compared to qPCR-based detection methods
- Fast and easy procedure with little hands on time

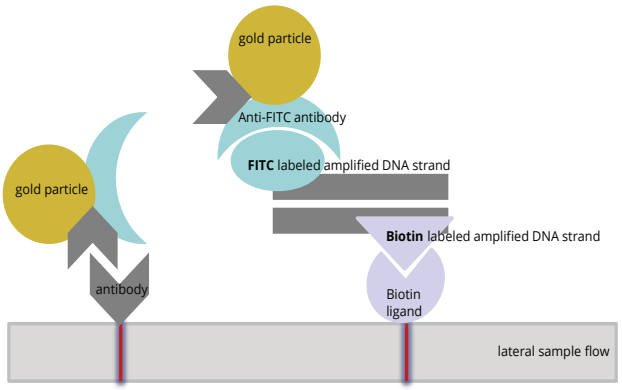
PRODUCT DETAILS

highQu PCRbeam™ Fast PCR Detection Kit is a convenient tool for fast detection of gene-specific amplification products obtained by PCR, LAMP or RPA. The detection is based on immunological reaction driven by Biotin and FITC (fluorescein isothiocyanate), thus the amplified DNA shall include Biotin and FITC labels. The PCR amplification has to be performed with one primer labeled with FITC at 5'-end and one primer labeled with Biotin at 5'-end. Alternatively the use of one of the labeled primers can be replaced by gene-specific FITC or Biotin labeled probe. Kit includes PCRbeam™ Membrane Strips that are coated with biotin-ligand on the test band and an anti-rabbit antibody on the control band. The bottom part of the strip which is used for sample application contains an anti-FITC antibody attached to gold particles. PCRbeam™ Detection Buffer is Tris-buffered saline enabling the detection.

The PCRbeam™ Fast PCR Detection Kit can be applied for established tests or home-brew assays as a fast and sensitive yes/no detection method. The detection sensitivity is up to 100 fold higher than the one achievable with ethidium bromide stained gels what provides an environment friendly save and economical alternative to the use of mutagen stains.

For establishing sensitive PCR-based tests before PCRbeam™ detection we recommend the use of hot-start PCR enzymes or master mixes, like highQu ALLin™ Hot Start Taq Mastermix or ALLin™ Hot Start Taq DNA Polymerase.

PRINCIPLE



control band
Gold particles that are not captured on the test band react with antibody attached to the control line on the membrane.

test band
PCR product amplified with 5'FITC-labeled and 5'Biotin-labeled primers binds to golden particles with attached anti-FITC antibody and to Biotin ligand on the membrane strip.

The membrane strip is soaked for 10 minutes into the vial with the detection buffer mixed with PCR product. The lateral sample flow driven by gold particles moves the solution up the strip. FITC labeled DNA strand binds with the anti-FITC antibody on the gold particle and Biotin labeled DNA strand is caught by Biotin ligand attached to the test band. As both DNA strands remain hybridized at room temperature, the test band builds an aggregate that develops red-blue color. Excess gold particles that were not caught by FITC move up the strip and the anti-FITC antibody binds to the anti-rabbit antibody to develop the red-blue colored control band. If there is no PCR product in the reaction, then only the control band will be visible. If there is a specific product, the test band will be colored as well.

NOTES

- Optimize and perform PCR with one primer labeled with FITC at 5'-end and one primer labeled with Biotin at 5'-end.
- Apply the PCRbeam™ Fast PCR Detection Kit only for established PCR assays, as a yes/no detection tool.
- Up to 5 pg DNA can be detected using PCRbeam™ Kit.
- Before starting the detection procedure warm the PCRbeam™ Membrane Strips and PCRbeam™ Detection Buffer at room temperature for 5 minutes.
- Avoid carrying over of the mineral oil when pipetting the PCR products for detection. Oil interferes with detection as it affects the lateral flow of the sample.

PROTOCOL

- Pipet 100 µl of the PCRbeam™ Detection Buffer into the plate or into the marked empty PCR vials.
- Add 5-10 µl of PCR product into each vial with detection buffer. Mix by gentle pipetting. Use up to 20 µl of the PCR mixture in case low yield is suspected.
- Insert the PCRbeam™ Membrane Strip into each vial so that the indicated spot for sample is soaked in the liquid.
- Incubate at room temperature for 2-10 min until the control band (if positive, the test band as well) gets red-blue color.
- Interpret the results immediately as yes (2 bands: control and test) or no (1 control band), independently on the intensity of the color of the bands.

25 mM and 10 mM dNTP Mixes & 100 mM dNTP Set

bulk quantities available

CAT.#	SIZE	COMPONENTS	COMPONENT COMPOSITION
dNTP Mixes:			
NUM0101	1 ml	1 ml - 25 mM dNTP Mix	Aqueous solution of equal concentration of 25 mM each of 4 dNTPs, pH 8.5 (4°C)
NUM0201	1 ml	1 ml - 10 mM dNTP Mix	Aqueous solution of equal concentration of 10 mM each of 4 dNTPs, pH 8.5 (4°C)
dNTP Sets:			
NUS0101	4 x 0.25 ml	0.25 ml - 100 mM dATP	Aqueous solution of 100 mM dATP, pH 8.5 (4°C)
		0.25 ml - 100 mM dCTP	Aqueous solution of 100 mM dCTP, pH 8.5 (4°C)
		0.25 ml - 100 mM dGTP	Aqueous solution of 100 mM dGTP, pH 8.5 (4°C)
		0.25 ml - 100 mM dTTP	Aqueous solution of 100 mM dTTP, pH 8.5 (4°C)
NUS0105	4 x 1 ml	1 ml - 100 mM dATP	Aqueous solution of 100 mM dATP, pH 8.5 (4°C)
		1 ml - 100 mM dCTP	Aqueous solution of 100 mM dCTP, pH 8.5 (4°C)
		1 ml - 100 mM dGTP	Aqueous solution of 100 mM dGTP, pH 8.5 (4°C)
		1 ml - 100 mM dTTP	Aqueous solution of 100 mM dTTP, pH 8.5 (4°C)

Storage: *In the dark at -20°C.*

APPLICATIONS

All molecular biology applications including dNTPs, like:

- cDNA synthesis
- Standard PCR, Long and high-fidelity PCR
- qPCR
- Sequencing

BENEFITS

- Highest quality, >99% HPLC pure dNTPs for high & reproducible yields
- Pure from DNA contamination and from PCR inhibitors
- Highly stable remain pure after weeks at room temperature, after 30 freezing thawing cycles and during the 40 PCR cycles
- Available in ready-to use mixes and sets for maximized flexibility

PRODUCT DETAILS

highQu dNTP sets and mixes meet all highest industry standards and allow for unrivaled performance of your PCR and other DNA synthesis reactions.
Produced under the stringent quality monitoring conditions, they guaranty reproducible results. More than 99% HPLC purity eliminates inhibitions of PCR and allows for increased yields with higher dNTP concentrations.

Exceptional stability eliminates dNTP usability concerns related to short term ambient temperature shipments, room temperature storage or PCR exceeding 40 cycles.

PREPARATION OF DNTP MIXES FROM A SET

- Highly concentrated solutions require thorough mixing before the use.
- The optimal dNTP mix shall have equal concentrations of all 4 dNTPs.
- To prepare from a set of 4 dNTPs mixes of common concentrations, follow the guidelines below:

Use same volume of each from four 100 mM dNTP solutions:	PCR Water	Resulting 1 ml Mix concentration:
20 µl	920 µl	2 mM dNTP
25 µl	900 µl	2.5 mM dNTP
100 µl	600 µl	10 mM dNTP
250 µl	-	25 mM dNTP

IN VITRO RESEARCH USE ONLY

PROTOCOL RECOMMENDATIONS FOR STANDARD PCR

- Typical concentration of each dNTP in the reaction is 0.2 - 0.25 mM. Higher concentration increase yields, however Mg^{2+} ions bind to dNTPs, therefore, both components shall be present in coordinated concentrations. Too high dNTPs and magnesium concentrations reduce PCR fidelity.
- Mix well each dNTP and magnesium solution, to avoid concentration fluctuations.
- Use final 3 mM $MgCl_2$ with 0.25 mM each dNTP concentration for routine PCR.

Starting dNTP Mix conc.	Vol. of dNTP mix in 50 µl r.	Final Mg^{2+} conc. in r.	Vol. of 50 mM $MgCl_2$ in 50 µl rxn to achieve desired conc.
10 mM	1.25 µl	2 mM	2 µl
25 mM	0.5 µl	3 mM	3 µl

PCR Water

bulk quantities available

CAT.#	SIZE	COMPONENTS	COMPONENT COMPOSITION
WAT0110	10 x 1 ml	10 x 1 ml - PCR Water	PCR-grade, nuclease free water
Storage:	*In the dark at -20°C.*	*To minimize the effects of the contamination during the use it is not recommended to store water at room temperature.*	

PRODUCT DETAILS

highQu PCR Water is a supplementary high quality reagent for all demanding applications. It saves time being on your bench and guaranties purity of reactions and inhibition-free performance of PCR reagents.

highQu PCR Water is a deionized, membrane filtered water continuously tested in ultrasensitive qPCR and PCR applications, in amplification of long targets and highly specific detection of few copies of templates.

APPLICATIONS

- All molecular biology applications

BENEFITS

- Pure PCR-grade, nuclease free water for excellence in molecular biology applications
- Same PCR Water is supplied with most of highQu products for maximum convenience and guaranteed performance
- Tested in most demanding PCR and qPCR applications

PROTOCOL

- Use PCR Water in all PCR applications like described in typical protocols.

IN VITRO RESEARCH USE ONLY

Ladders and Stains for DNA and Protein Electrophoresis

Selection of DNA Electrophoresis Ladders *page 71*

DNA LADDER	LADDER BANDS IN BP AND IN KB, ALL REFERENCE BANDS IN BOLD
Take5™ 50 bp	50 - 100 - 150 - **200** - 250 - 300 - 350 - 400 -- **500** - 600 - 700 - 800 - 900 - 1 - 1,2 -- 1,5
Take5™ 100 bp	----- 100 ---------- 200 -------- 300 --------- 400 -- **500** - 600 - 700 - 800 - 900 - 1 --------- **1,5** ---------- 3
Take5™ 1kb	---- 100 ---------- 200 -------- 300 --------- 400 -- **500** - 600 - 700 - 800 - 900 - 1 --------- **1,5** - 2 - 2,5 - **3** - 4 - 5 - 6 - 8 - 10
Take5™ HR	---------- 250 ---------- 500 ---------- 750 ---------- **1** --------- 1,5 - 2 - 2,5 - **3** - 4 - 5 - 6 - 8 - 10 - 25

Selection of DNA Electrophoresis Stains *page 72-73*

FEATURES OF NUCLEIC ACID STAINS	StainIN™ RED Nucleic Acid Stain *page 72*	StainIN™ GREEN Nucleic Acid Stain *page 73*
Fluorescence	Red	DNA green, RNA red
Excitation max.	540 nm	490 nm
Emission max.	630 nm	520 nm and 635 nm
In gel staining during agarose electrophoresis	Yes	Yes
Staining of PAGE during electrophoresis	Yes	Yes
Post-run staining	-	-
DNA detection sensitivity	0,3 - 0,6 ng	0,1 - 0,3 ng
UV detection	Yes	Yes
Blue light detection	-	Yes
Used as loading dye	-	-
Cloning compatible	Yes, when UV exposure is minimal	Yes, under Blue light
Filters to use	Ethidium Bromide	SYBR® Green

DNA Electrophoresis Ladders

CAT.#	SIZE	COMPONENTS
DNL0102	200 appl.	2 x 0.5 ml - Take5™ 1 kb DNA Ladder 2 x 1 ml - Take5™ Loading Dye, 6X
DNL0202	200 appl.	2 x 0.5 ml - Take5™ 100 bp DNA Ladder 2 x 1 ml - Take5™ Loading Dye, 6X
DNL0302	200 appl.	2 x 0.5 ml - Take5™ 50 bp DNA Ladder 2 x 1 ml - Take5™ Loading Dye, 6X
DNL0402	200 appl.	2 x 0.5 ml - Take5™ HR DNA Ladder 2 x 1 ml - Take5™ Loading Dye, 6X

COMPONENT COMPOSITION

Ready to load ladders contain highly purified PCR products combined with plasmid digests, they are supplied in 1x loading dye: 10 mM Tris-HCl (pH 8.0) 10 mM EDTA, glycerol and tracking dyes.
6X Take5™ Loading Dye includes 10 mM Tris-HCl (pH 8.0) 60 mM EDTA, glycerol and three electrophoresis tracking dyes (Xylene cyanol FF, Bromophenol blue, Orange G).
1 µl of 6X dye shall be used for 5 µl of DNA sample, mixed well and loaded.

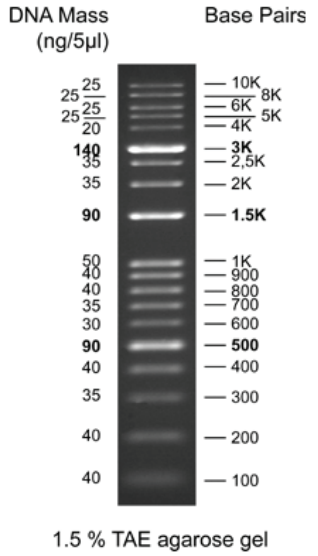
IN VITRO RESEARCH USE ONLY

APPLICATIONS

- DNA size determination and approximate DNA quantification on agarose gels

BENEFITS

- Room-temperature-stable, always ready to be used
- Sharp bands, bright reference bands, indicated DNA mass
- Take5™ ladders are supplied with loading dye for DNA samples



1.5 % TAE agarose gel

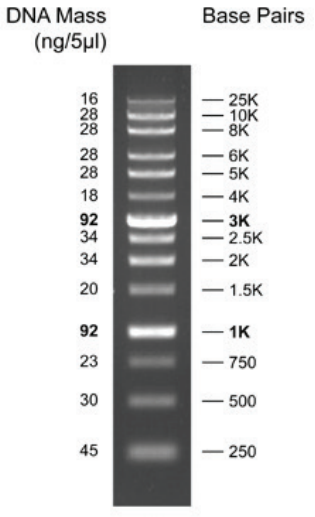
Take5™ 1kb DNA Ladder

100 bp- 10 kb Range
0.5; 1.5 & 3 kb Reference
19 Bands
172 ng/µl Concentration

Ready-to-use:
Load: 5 µl/well

Tracking Dyes:

- Xylene cyanol FF (~4 kb)
- Bromophenol blue (~0.4 kb)
- Orange G (<50 bp)



0.7 % TAE agarose gel

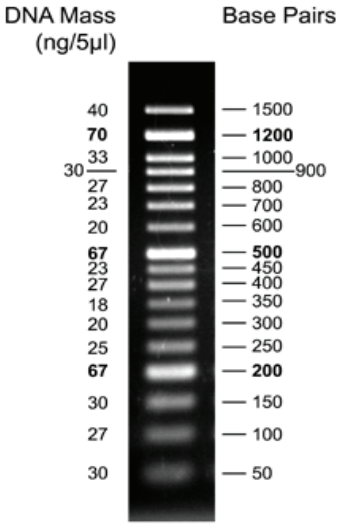
Take5™ HR DNA Ladder

250 bp- 25 kb Range
1 & 3 kb Reference
14 Bands
104 ng/µl Concentration

Ready-to-use:
Load: 5 µl/well

Tracking Dyes:

- Xylene cyanol FF (~4 kb)
- Bromophenol blue (~0.4 kb)



2% TAE agarose gel

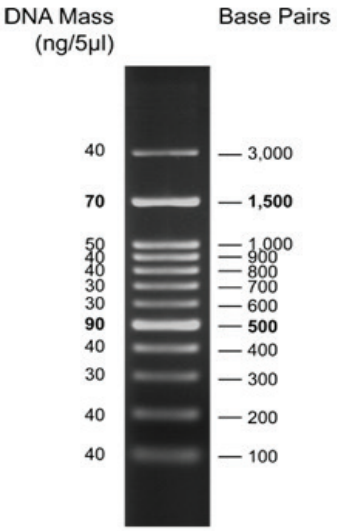
Take5™ 50 bp DNA Ladder

50 bp- 1.5 kb Range
0.2 & 0.5 kb Reference
17 Bands
112 ng/µl Concentration

Ready-to-use:
Load: 5 µl/well

Tracking Dyes:

- Orange G (<50 bp)



1.5 % TAE agarose gel

Take5™ 100 bp DNA Ladder

100 bp- 3 kb Range
0.5 & 1.5 kb Reference
12 Bands
108 ng/µl Concentration

Ready-to-use:
Load: 5 µl/well

Tracking Dyes:

- Xylene cyanol FF (~4 kb)
- Orange G (<50 bp)

Stable: Room temperature - 6 months; at +4°C - 12 months; at -20°C - 24 months (or until expires)

StainIN™ RED Nucleic Acid Stain

CAT.#	SIZE	COMPONENTS	COMPONENT COMPOSITION
NAS0101	1 ml	1ml - StainIN™ RED Nucleic Acid Stain	Red DNA and RNA stain in diluted DMSO, as 20000 X solution to be used at 1 X concentration in agarose or polyacrylamide gels and at 0,5 X concentration in electrophoresis buffers.

Storage: *In the dark at +4°C.*

Disposal: *Used dye solutions or melted gels shall be run through filters and later disposed with plenty of water down the drain.*

APPLICATIONS

- Staining of NA in agarose and polyacrylamide gels during electrophoresis for ssDNA, dsDNA and RNA visualization and gel documentation under the UV light

BENEFITS

- Much more safe alternative to ethidium bromide
- Highly sensitive NA detection - up to 2x more sensitive than EtBr
- Time saving – in gel stain, no post- run staining, no destaining

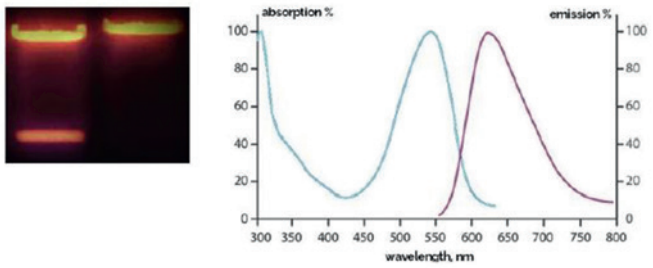
PRODUCT DETAILS

StainIN™ RED Nucleic Acid Stain is a significantly safer alternative to ethidium bromide. It is same easy to use, twice as sensitive and much more secure. At least twice as economical as competing products, this novel stain can be also used and disposed with less environmental and health concerns compared to ethidium bromide.

StainIN™ RED is a fluorescent dye that allows detection of >0,3 ng of DNA in both agarose and polyacrylamide gels. It binds to both ds DNA, ssDNA and RNA and emits red fluorescence detectable under the UV light and documented with same filters as ethidium bromide. For cloning applications, UV exposure shall be minimized.

Much smaller than ethidium bromide carcinogenicity of the dye has been proved by Ames-test. Mammalian cell mutagenicity tests, both mouse marrow erythrocyte micronucleus and spermatocyte chromosomal aberration tests gave negative mutagenicity results.

PERFORMANCE



Left image - agarose gel stained with StainIN™ RED Nucleic Acid Stain.
Right image - StainIN™ RED excitation maxima - 540 nm, emission - 630 nm.

PROTOCOL FOR AGAROSE GEL ELECTROPHORESIS

1. Wear gloves when working with all NA stains, buffers and gels.
2. Prepare the agarose gel solution like recommended by supplier.
3. Cool down the agarose after boiling to a hand-friendly temperature.
4. Add 5µl of StainIN™ RED solution per 100 ml of the gel right before casting the gel.
5. Mix the gel solution very gently to distribute the dye but not to produce air bubbles. Pour the gel, insert the combs.
6. Prepare the required volume of 1X TAE or 1X TBE buffer to be used in the electrophoresis tank.
7. Add 2,5 - 3 µl of StainIN™ RED solution per 100 ml of the 1X electrophoresis running buffer.
8. Add both gel and the buffer into the electrophoresis tank and run electrophoresis like usual.
9. Visualize nucleic acids under the UV light.

- Destaining is not needed, but it might help to reduce the background; post-run staining is not recommended.
- Use Ethidium bromide filters for gel photography.
- After a few runs refresh the electrophoresis buffer in electrophoresis tank prepared as in step 7.
- If you reuse the gel, add at least half a portion of the stain each time after boiling and cooling the gel solution down (like in step 4).

PROTOCOL FOR PAGE

1. Wear gloves when working with all NA stains, buffers and gels.
2. Prepare the native or denaturing PAA gel ike recommended by supplier.
3. Add TEMED and APS and proceed to the next step immediately.
4. Add 5µl of StainIN™ RED solution per 100 ml of the gel right before casting the gel.
5. Mix the gel solution very gently to distribute the dye but not to produce air bubbles. Pour the gel, insert the combs.
6. Prepare the required volume of 1X TBE buffer to be used in the electrophoresis tank.
7. Add 2,5 - 3 µl of StainIN™ RED solution per 100 ml of 1X running buffer.
8. Add both gel and the buffer into the electrophoresis tank and run electrophoresis like usual.
9. Visualize nucleic acids under the UV light.

- Destaining is not needed, post-run staining is not recommended.
- Use Ethidium bromide filters for gel photography.
- After a few runs refresh the electrophoresis buffer in electrophoresis tank prepared as in step 7.

IN VITRO RESEARCH USE ONLY

StainIN™ GREEN Nucleic Acid Stain

CAT.#	SIZE	COMPONENTS	COMPONENT COMPOSITION
NAS0201	1 ml	1ml - StainIN™ GREEN Nucleic Acid Stain	Aqueous solution of green DNA and RNA stain, as 20000 X solution to be used at 1 X concentration in agarose or polyacrylamide gels and at 0,5 X concentration in electrophoresis buffers.

Storage: *In the dark at +4°C.*

Disposal: *Used dye solutions or melted gels shall be run through filters and later disposed with plenty of water down the drain.*

APPLICATIONS

- Staining of NA in agarose and polyacrylamide gels during electrophoresis for ssDNA, dsDNA and RNA visualization and gel documentation
- UV or Blue LED detection, excellent for cloning applications

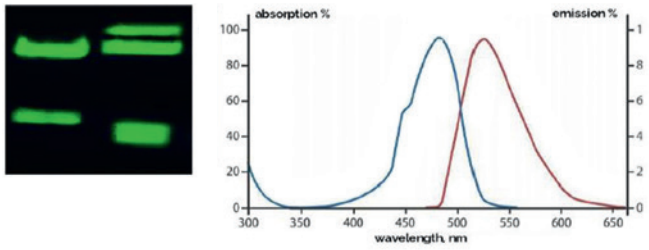
BENEFITS

- Much more safe alternative to ethidium bromide, more economical alternative to competing green dyes
- Unique – two emission peaks, colors DNA in green, RNA in red
- Highly sensitive NA detection - up to 4x more sensitive than EtBr
- Time saving – in gel stain, no post- run staining, no destaining

PRODUCT DETAILS

StainIN™ GREEN Nucleic Acid Stain is a significantly safer alternative to ethidium bromide. It is same easy to use, four times as sensitive and much more secure. Twice as economical as competing green dyes, this novel stain can be also used and disposed with less environmental and health concerns compared to ethidium bromide. It is a fluorescent dye that allows detection of >0,1 ng of DNA in both agarose and PAA gels. It binds to both dsDNA, ssDNA and RNA and emits green fluorescence when bound to DNA and red fluorescence when bound to RNA detectable under the UV or Blue light and documented with same filters like other green dyes. StainIN™ GREEN is ideal for DNA extraction from gels for cloning. Much smaller than ethidium bromide carcinogenicity of the dye has been proved by Ames-test. Mammalian cell mutagenicity tests, mouse marrow erythrocyte micronucleus and spermatocyte chromosomal aberration tests gave negative mutagenicity results.

PERFORMANCE



Left image - agarose gel stained with StainIN™ GREEN Nucleic Acid Stain.
Right image - StainIN™ GREEN excitation maxima - 490 nm,
emission maximas – bound to DNA - 520 nm; bound to RNA - 635 nm (not shown here).

PROTOCOL FOR AGAROSE GEL ELECTROPHORESIS

1. Wear gloves when working with all NA stains, buffers and gels.
2. Prepare the agarose gel solution like recommended by supplier.
3. Cool down the agarose after boiling to a hand-friendly temperature.
4. Add 5µl of StainIN™ GREEN solution per 100 ml of the gel right before casting the gel.
5. Mix the gel solution very gently to distribute the dye but not to produce air bubbles. Pour the gel, insert the combs.
6. Prepare the required volume of 1X TAE or 1X TBE buffer to be used in the electrophoresis tank.
7. Add 2,5 - 3 µl of StainIN™ GREEN solution per 100 ml of the 1X running buffer.
8. Add both gel and the buffer into the electrophoresis tank and run electrophoresis like usual.
9. Visualize nucleic acids under Blue light or UV (~500-650 nm).

- Destaining is not needed, but it might help to reduce the background; post-run staining is not recommended.
- Use only Blue light if you intend to clone the DNA.
- Use SYBRGreen filters for gel photography.
- After a few runs refresh the electrophoresis buffer in electrophoresis tank prepared as in step 7.
- If you reuse the gel, add at least half a portion of the stain each time after boiling and cooling the gel solution down (like in step 4).

PROTOCOL FOR PAGE

1. Wear gloves when working with all NA stains, buffers and gels.
2. Prepare the native or denaturing PAA gel like recommended by supplier.
3. Add TEMED and APS and proceed to the next step immediately.
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8. Add both gel and the buffer into the electrophoresis tank and run electrophoresis like usual.
9. Visualize nucleic acids under Blue light or UV (~500-650 nm).

- Destaining is not needed, post-run staining is not recommended.
- Use SYBRGreen filters for gel photography.
- After a few runs refresh the electrophoresis buffer in electrophoresis tank prepared as in step 7.

IN VITRO RESEARCH USE ONLY

Selection of Protein Electrophoresis Ladders *page 75*

PROTEIN LADDER	COLORED LADDER BANDS IN KDA (TRIS-GLYCINE, 4-20% GRADIENT GEL)
Cozy™ Prestained	----- 11 -- 17 ------- **25** -- 35 -- 48 -- 63 -- **75** -- 100 -- 135 --- 180
CozyHi™ Prestained	5 --- 11 - 17 --20-- **25** -- 35 -- 48 -- 63 -- **75** -- 100 -- 135 --- 180 ---- 245
CozyXL™ Prestained	----- 11 -- 17 ------- **25** -- 35 -- 48 -- 63 -- **75** -- 100 --135 --- 180 ----- 245 --- **310**

Protein Electrophoresis Ladders

CAT.#	SIZE	COMPONENTS	COMPONENT COMPOSITION
PRL0102	200 appl.	2 x 0.5 ml - Cozy™ Prestained Protein Ladder	Ready to load ladders contain highly purified prestained proteins, supplied denatured in 20 mM Tris-Phosphate (pH 7.5 at 25°C), 2% SDS, 0.2 mM DTT, 3.6 M Urea, 15% (v/v) glycerol).
PRL0202	200 appl.	2 x 0.5 ml - CozyHi™ Prestained Protein Ladder	
PRL0302	200 appl.	2 x 0.5 ml - CozyXL™ Prestained Protein Ladder	IN VITRO RESEARCH USE ONLY

APPLICATIONS

- Approximate protein molecular weight determination on denaturing gels and Western blots
- Monitoring of electrophoresis process and transfer efficiency

BENEFITS

- Room-temperature-stable, always ready to be used
- Sharp bands, bright colors

Cozy™

11 - 180 kDa Range
25 & 75 kDa References
10 Bands; 0.2-0.4 µg/µl each

CozyHi™

5 - 245 kDa Range
25 & 75 kDa References
13 Bands; 0.2-0.4 µg/µl each

CozyXL™

11 - 310 kDa Range
25 & 75 kDa References
12 Bands; 0.2-0.4 µg/µl each

Images taken after **Tris-Glycine 4-20% gel** electrophoresis show guidelines for approximate protein MW estimation. Given molecular weight of each protein is very approximate. It has been determined by callibrating it against unstained protein of same size. For precise sizing, such calibration shall be done exactly at conditions used.

PROTOCOLS

Ready-to-use prestained protein ladders:

- Thaw if needed, mix and load: 3- 5 µl/gel well for electrophoresis
- Load: 3- 5 µl/gel well for Western transfers
- For Western transfer, use 100 V, 90 minutes. To see high MW bands better, perform slower overnight transfer.
- Transfer Buffer: 25 mM Tris, 192 mM glycine, 20% methanol.

Stability under certain storage conditions:

- Room temp. at least 2 weeks
- +4°C at least 3 months
- -20°C at least until expiry date, printed on the label

PRODUCT USE LIMITATIONS

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professionally simple

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