Bio-Works

DATA SHEET

WorkBeads 40/1000 ACT
WorkBeads 40/10 000 ACT
GoBio Mini ACT

WorkBeads™ 40/1000 ACT and WorkBeads 40/10 000 ACT are pre-activated resins that enable easy and reliable coupling of proteins, peptides, and low-molecular weight substances for the preparation of customized chromatography resins or enzyme reactors. The bromohydrin active group reacts with thiol, amino and hydroxyl groups. Two different resin porosities are available to facilitate optimized coupling of ligands of different sizes, or to optimize the prepared affinity resin for target molecules of different sizes. The ready-to-use GoBio™ Mini ACT columns are prepacked with WorkBeads 40/1000 ACT resin and are available in two column sizes: 1 mL and 5 mL.

- Easy and reliable coupling procedure
- Stable covalent linkage
- Suitable for coupling of ligands containing thiol, amino and hydroxyl groups

Resin description

WorkBeads are agarose-based chromatographic resins manufactured using a proprietary method that results in porous beads with a tight size distribution and exceptional mechanical stability. Agarose based matrices have been successfully used for decades in biotechnology, from research to production scale purification, due to their exceptional compatibility with biomolecules including proteins, peptides, nucleic acids, and carbohydrates. WorkBeads resins are designed for separations that require optimal capacity and purity.

WorkBeads 40/1000 ACT and WorkBeads 40/10 000 ACT pre-activated resins are a reliable starting material for the preparation of customized chromatography resins. A wide number of organic molecules and biomolecules containing thiol- (i.e., sulfhydryl), amino- or hydroxyl-groups can be coupled covalently by nucleophilic displacement to the agarose matrix activated according to the well-documented bromohydrin method, see Figure 1.

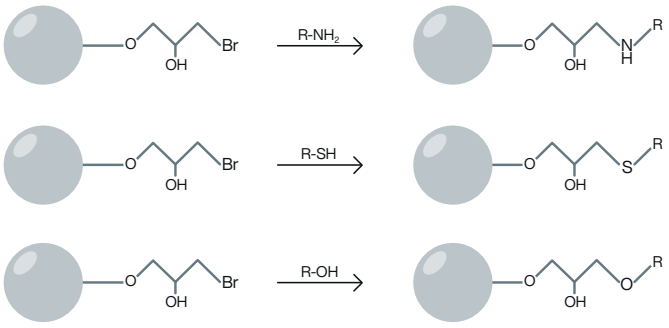

Figure 1. Reaction scheme for coupling a, from top to bottom, primary amine, thiol, and alcohol to bromohydrin activated resin.

GoBio Mini column description

The column is made from biocompatible polypropylene which does not significantly interact with biomolecules. The top and bottom filters are made from polyethylene. The ready-to-use GoBio Mini columns are delivered with an inlet plug, a cut-off outlet, and a cap for storage. The columns can be connected to a syringe, pump or chromatography system using finger tight fittings (coned 10 – 32) for 1/16" o.d. tubing (standard HPLC PEEK tubing

The main characteristics of the pre-activated WorkBeads resins and GoBio Mini ACT are shown in Tables 1 and 2. For additional information about the derivatization of pre-activated resins, see instructions IN 40 400 010 and IN 45 400 010.

Applications

The design of customized chromatography resins often requires the use of methods for covalent coupling of a ligand to the matrix (non-functionalized resin). Coupling is generally done in three steps: activation, coupling and blocking of unused activated groups. WorkBeads 40/1000 ACT and WorkBeads 40/10 000 ACT are pre-activated to have a bromohydrin group that is reactive towards primary amines, thiol- (sulfhydryl), hydroxyl-, or histidyl residues. Most substances containing one or more of these groups can be coupled to the resin. The coupling reaction results in a stable covalent bond. The blocking step is required to eliminate further coupling of substances that are in contact with the resin during subsequent use of the prepared resin. Blocking is often done using ethanolamine or ß-mercaptoethanol.

The bromohydrin coupling method does not introduce additional charges. The coupling is done at room temperature in aqueous solution and does not release hazardous chemicals during normal use. The work can be performed on the lab bench if the substance to be coupled is not hazardous.

Ligands with free amino and sulfhydryl groups will couple easily overnight in basic pH conditions at room temperature, with constant stirring to keep the resin in suspension. Coupling of substances containing hydroxyl groups require high pH (pH > 12) due to the low nucleophilicity of this functionality. The hydroxyl groups need to be deprotonated. Coupling of thiol-containing substances can be done under weakly alkaline conditions.

In general, the coupling yield will increase at higher pH. However, hydrolysis of the bromohydrin groups will compete with the coupling reaction at high pH values. An optimum value for pH is often observed for coupling yield. Users should develop a specific procedure optimized for the coupling reaction and for the stability of the substance to be immobilized.

WorkBeads 40/1000 ACT and WorkBeads 40/10 000 ACT are suitable for the preparation of affinity chromatography resins or enzyme reactors. The porosity of these two resins differs, see "Exclusion limit" in Table 1. A higher porosity for the prepared affinity resin may be required if the ligand to be attached or the target is large. A lower porosity can be used if the ligand or the target is small.

Table 1. Main characteristics of WorkBeads 40/1000 ACT and WorkBeads 40/10 000 ACT resins.

	WorkBeads 40/1000 ACT	**WorkBeads 40/10 000 ACT**
Target substance	Small molecules and peptides	Small molecules, peptides, proteins, e.g., Immunoglobulins
Target groups	Thiol, amino, and hydroxyl groups	Thiol, amino, and hydroxyl groups
Matrix	Rigid, highly cross-linked agarose	Rigid, highly cross-linked agarose
Average particle size (D_{V50})[1]	45 µm	45 µm
Reactive groups	Bromohydrin	Bromohydrin
Exclusion limit	1×10^6 Da (globular proteins)	10×10^6 Da (globular proteins)
Max flow rate[2]	600 cm/h	600 cm/h
Reactive groups content	200 µmol/mL	200 µmol/mL
Chemical stability (before coupling[3])	Buffers pH < 8.5	Buffers pH < 8.5
Chemical stability (after coupling[4])	Compatible with all standard aqueous buffers used for protein purification, 1 M NaOH, 30% isopropanol or 70% ethanol. Should not be stored at < pH 3 for prolonged time	
pH stability[4]	2 – 13 (after coupling)	2 – 13 (after coupling)
Storage[5]	2 to 25 °C in 20 % ethanol	2 to 25 °C in 20 % ethanol

1 The median particle size of the cumulative volume distribution.
2 Determined in water using a 10 × 300 mm column. Note: When doing a purification, the optimal flow rate during binding is depending on the sample.
3 Avoid substances containing thiol and amino groups. Substances containing hydroxyl groups will only react if deprotonated. The unreacted resin is generally stable in alcohols at neutral pH.
4 Agarose matrix and linker. Stability of the coupled substance may differ.
5 The choice of storage conditions for the coupled resin depends on the nature of the ligand.

Table 2. Main characteristics of and GoBio Mini ACT columns.

	GoBio Mini ACT
Resin	WorkBeads 40/1000 ACT
Matrix	Rigid, highly cross-linked agarose
Average particle size (D_{V50})[1]	45 µm
Reactive group	Bromohydrin
Reactive-groups content	200 µmol/mL resin
Column volume	1 mL 5 mL
Column dimension	7 × 28 mm (1 mL) 13 × 38 mm (5 mL)
Recommended flow rate[2] GoBio Mini 1 mL GoBio Mini 5 mL	 0.25 – 1 mL/min (37 – 150 cm/h) 1.25 – 5 mL/min (56 – 225 cm/h)
Maximum flow rate[3] GoBio Mini 1 mL GoBio Mini 5 mL	 5 mL/min (780 cm/h) 20 mL/min (900 cm/h)
Maximum back pressure	0.3 MPa, 3 bar, 43 psi
Chemical stability (before coupling[4])	Buffers pH < 8.0
Chemical stability (after coupling[5])	Compatible with all standard aqueous buffers used for protein purification, 1 M NaOH, 30% isopropanol or 70% ethanol. Should not be stored at < pH 3 for prolonged time.
pH stability[5]	2 – 13 (after coupling)
Storage[6]	2 to 25°C in 20% ethanol (before coupling)

[1] The median particle size of the cumulative volume distribution.
[2] Optimal flow rate during binding is depending on the sample. During column wash and elution, a flow rate of 1 mL/min and 5 mL/min can be used for 1 mL and 5 mL columns, respectively. Note: The maximum pressure the packed bed can withstand depends on the sample/liquid viscosity and chromatography resin characteristics. The pressure also depends on the tubing used to connect the column and the system restrictions after the column outlet.
[3] Aqueous buffers at 20°C. Decrease the maximum flow rate if the liquid has a higher viscosity. Higher viscosities can be caused by low temperature (use half of the maximum flow rate at 4°C), or by additives (e.g. use half of the maximum flow rate for 20% ethanol).
[4] Avoid substances containing thiol and amino groups. Substances containing hydroxyl groups will only react if deprotonated. The unreacted resin is generally stable in alcohols at neutral pH.
[5] Agarose matrix and linker. Stability of the coupled substance may vary.
[6] The choice of storage conditions for the coupled resin depends on the nature of the ligand. Often 20% ethanol can be used as a bacteriostatic agent.

Standard coupling protocol using bulk resin

Coupling conditions are listed in Table 3.

1. Wash the resin with deionized water on a glass filter, and dry using suction until drops stop coming.
2. Dissolve the ligand to be coupled in a suitable coupling buffer, or in water.
3. Add the ligand solution to the resin in a final resin slurry concentration of 40-60%.
4. Incubate overnight at room temperature with stirring.
5. Wash with coupling buffer or deionized water to remove unreacted ligand. Suction dry the resin.
6. Block the remaining reactive groups by incubation at room temperature overnight with suitable blocking reagent, for example 1 M ethanolamine-HCl, pH 9.5, with stirring.
7. Wash with coupling buffer or deionized water to remove unreacted blocking reagent.
8. If the resin is not being used for the intended application immediately transfer it into 20% ethanol for storage. The resin can be packed in a chromatography column or be used as a suspension.

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Standard coupling protocol using GoBio Mini ACT

Note: GoBio Mini ACT is prepacked with WorkBeads 40/1000 ACT.

Coupling conditions are listed in Table 3.

1. Dissolve the ligand to be coupled in a suitable coupling buffer, or in water.
2. Wash the column with suitable coupling buffer, or in water.
3. Add the ligand solution to the column. If the ligand solution has the same volume as the column this is easily done using a syringe. If the ligand solution volume is larger than the column volume the solution can be re-circulated using a pump.
4. Allow time for the reaction to take place. The reaction time is ligand dependent and needs to be optimized. A general recommendation is to leave the reaction to take place overnight at room temperature (approximately 16 hours).
5. Wash the column with coupling buffer or deionized water to remove unreacted ligand.
6. Block the remaining reactive groups by incubation at room temperature overnight with suitable blocking reagent. For example, by using 1 M ethanolamine-HCl, pH 9.5.
7. Wash the column with coupling buffer or deionized water to remove unreacted blocking reagent.
8. If the column is not quickly used for the intended application immediately equilibrate with 5 CV of storage solution (e.g., 20% ethanol) or a suitable buffer. Close the column using the cap and plug.

For more detailed coupling instructions, please refer to instructions IN 40 400 010 (bulk) and IN 45 400 010 (GoBio Mini ACT) available on www.bio-works.com

Cleaning-in-Place (CIP)

When the ligand-coupled resin is used for purification or in an enzyme reactor contaminant from the sample (feed), e.g., cell debris, lipids, nucleic acids and protein precipitates, may gradually build up in the resin. The severity of this fouling process depends on the type of sample applied to the column, and the pre-treatment of the sample. This contamination may reduce the performance of the column over time. Regular cleaning (CIP) keeps the resin clean, reduces the rate of further contamination, and prolongs the capacity, resolution, and flow properties of the column.

A specific cleaning protocol should be designed for each process according to the type of sample purified and the stability of the ligand attached to the resin. For stable resins, cleaning can often be done overnight with 1 M NaOH, whereas resins with sensitive ligands can often be cleaned using non-ionic detergent.

Storage

Store at 2 to 25°C in 20% ethanol.

Close GoBio Mini columns securely using the plug and cap included.

The choice of storage conditions and the stability of the coupled resin depend on the nature of the coupled ligand.

Table 3. Type of ligand and most suitable coupling conditions.

Type of ligand	Functional group of ligand	Coupling conditions
Organic molecules, peptides	Thiol (Sulfhydryl) (-SH)	pH > 7 and higher
Organic molecules, peptides	Amino[1] (-NH_2, -NH, -N)	pH > 7 and higher[2]
Proteins, polypeptides	Thiol (Sulfhydryl) (-SH)	pH 7 and higher
Proteins, polypeptides	Primary amino (-NH_2)	Carbonate buffer pH 8 – 8.5[3]
Substance stable at high pH	Hydroxyl (-OH)	pH > 12[4,5]

[1] Substances containing primary, secondary, and tertiary amines.
[2] Alkaline ligands used in excess may give high enough pH for the reaction to take place. Dissolve it in distilled water and let the basicity of the ligand determine the coupling pH.
[3] Sufficient coupling without denaturation of sensitive polypeptides and proteins. Coupling reaction at a lower temperature is also possible.
[4] High pH is required due to the low nucleophilicity of the hydroxyl group.
[5] At this pH hydrolysis of the bromohydrin will compete with the coupling reaction.

Related products

Product name	Pack size[1]	Article number
Prepacked columns		
GoBio Mini Dsalt 1 mL	1 mL × 5	45 360 103
GoBio Mini Dsalt 5 mL	5 mL × 5	45 360 107
GoBio Prep 16x100 Dsalt[2]	20 mL × 1	55 700 021
GoBio Prep 26x100 Dsalt	53 mL × 1	55 700 031
Bulk resins		
WorkBeads Dsalt	300 mL	40 360 003

[1] Other pack sizes can be found in the complete product list on www.bio-works.com
[2] Packed on request.

Ordering information

Product name	Pack size	Article number
Prepacked columns		
GoBio Mini ACT 1 mL[1]	1 mL × 1 1 mL × 5 1 mL × 10	45 400 001 45 400 003 45 400 004
GoBio Mini ACT 5 mL[1]	5 mL × 1 5 mL × 5 5 mL × 10	45 400 005 45 400 007 45 400 008
Bulk resins		
WorkBeads ACT 40/1000 ACT	50 mL 300 mL 1 L 5 L	40 400 001 40 400 003 40 400 010 40 400 050
WorkBeads 40/10 000 ACT	50 mL 300 mL 1 L 5 L	40 450 001 40 450 003 40 450 010 40 450 050

[1] GoBio Mini ACT columns are prepacked with WorkBeads 40/1000 ACT.

Orders: sales@bio-works.com or contact your local distributor.

For more information about local distributor and products visit www.bio-works.com or contact us at info@bio-works.com

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Bio-Works, Virdings allé 18, 754 50 Uppsala, Sweden. For local office contact information, visit bio-works.com/contact.

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