Protection of protein A resins during mAb purifications

Today, monoclonal antibodies (mAbs) represent the largest and most progressive biopharmaceutical market. The mAbs used for clinical applications are subject to very high purity requirements and there is thus a demand for more efficient and economical purification processes. Chromatography using protein A resins results in mAbs with high yield and purity and is therefore a key step in most mAb purification processes. The mAb-containing cell supernatant is often loaded directly onto the protein A column without prepurification, causing extensive bioburden on the resin. To increase the lifetime of the protein A resin and to improve the final purity of the mAb, we have included an upstream multimodal AIEX column (WorkBeads™ 40 TREN) in flow-through mode. This procedure removed 95% of the host cell proteins and 99% of the host cell DNA. The extensive removal of host cell impurities protected the protein A resin and resulted in a mAb product of improved purity.

Monoclonal antibodies

Monoclonal antibodies (mAbs) are genetically identical antibody copies used primarily in therapy and diagnostics. The main therapy areas for mAbs include cancer, inflammatory and autoimmune diseases, and viral infections. The global mAbs market accounted for approximately USD 100 billion in 2017 with an expected continued growth over the coming years. Most of the therapeutic antibodies are produced *in vivo*, *i.e*, they are overexpressed in a biological system. This puts a high demand on the downstream process. A challenging aspect of treatment with mAb drugs is the common requirement for frequent and high doses (up to grams of mAbs per year per patient) which in turn lead to corresponding levels of drug-related impurities. Even small traces of impurities may cause big cumulative effects. The purity requirements are therefore extremely stringent.

mAb purifications

Today the most widely used purification technique is affinity chromatography using protein A resins. This technique gives high yield of antibodies with a purity of more than 95%. For therapeutic use however, downstream chromatographic polishing steps are necessary to further enhance the purity by reducing process-related impurities such as host cell proteins (HCP), host cell DNA (HCD) and viruses to the very low levels required. The polishing steps should also remove any protein A leached from the protein A resin (ligand leakage) and aggregates which can be toxic in a pharmaceutical product.

WorkBeads affimAb

WorkBeads affimAb is an alkali-stable protein A resin from Bio-Works designed for mAb purification (Table 1). This high-capacity resin allows a higher purity of eluted mAbs from cell supernatants than the market-leading resin. The extraordinary high binding capacity, even at high flow rates (short residence times), allows high productivity in downstream bioprocesses.

Bioburden on protein A resins

Purification of antibodies or Fc-fusion proteins from mammalian host cells, such as Chinese Hamster Ovary (CHO), which today is the primary mAb expression system, results in extensive bioburden on the protein A column in the form of nucleic acids and protein impurities. The host cell nucleic acids together with host cell proteins in general, cause damage to the protein A resin. Regular cleaning-in-place (CIP) is mandatory in the purification process, but accumulative fouling of the packed column will still occur. Maximized lifetime of the protein A resin is thus an important requirement during the purification process development. It would be desirable to add a purification step before the protein A purification step in order to reduce the impurities that constitute the worst threat to the resin, and also in the process improve the final purity of the target antibody. Here, we apply upstream WorkBeads 40 TREN for pretreatment to protect the protein A resin from the heavy bioburden caused by the feed.

Table 1. Main characteristics of WorkBeads affimAb.

WorkBeads affimAb	
Target substance	Antibodies (IgG), bound via the F_c-region
Matrix	Rigid, highly cross-linked agarose
Average particle size[1] (D_{v50})	50 µm
Ligand	Recombinant protein A expressed in *E. coli* using animal-free medium
Dynamic binding capacity[2] (DBC)	> 40 mg human IgG/mL resin
Maximum recommended flow rate[3,4]	300 cm/h
Chemical stability	Compatible with all standard aqueous buffers used for protein purification and with 10 mM HCl (pH 2), 0.5 M NaOH (pH 12), 0.1 M sodium citrate buffer (pH 3), 6 M guanidine-HCl, and 20% ethanol. Should not be stored at low pH for prolonged time.
pH stability	3 - 10
Cleaning-in-place stability	Up to 0.5 M NaOH
Storage	2 to 8 °C in 20 % ethanol

[1]The median particle size of the cumulative volume distribution.
[2]DBC was determined at 10% breakthrough ($Q_{B10\%}$) by frontal analysis with 1 mg/mL human polyclonal IgG in PBS, pH 7.4 at 1.4 mL/min (240 cm/h, 2.5 minutes residence time) in a column packed with WorkBeads affimAb, column bed 6.6 x 100 mm.
[3]Maximum recommended flow rate at 20 °C using aqueous buffers. 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 when operating at 4 °C), or by additives (e.g., use half of the maximum flow rate for 20% ethanol).
[4]Maximum recommended flow rate determined in a 25 x 200 mm column

WorkBeads 40 TREN

WorkBeads 40 TREN is a multimodal anion exchange chromatography (AIEX) resin with a ligand based on Tris(2-aminoethyl)amine, TAEA, that is positively charged below pH 9. This ligand introduces both ionic and hydrophobic interactions giving the resin a unique selectivity.

The resin which is also high salt tolerant is effective in removal of most host cell impurities is thus an excellent choice for pretreatment in mAb purifications.

Table 2. Properties of WorkBeads 40 TREN

WorkBeads 40 TREN	
Target substances	Proteins, peptides, viruses, oligonucleotides
Average particle size[1] (D_{v50})	45 µm
Ionic group (ligand)	Tris(2-ethylaminoethyl) amine (TAEA)
Dynamic binding capacity (DBC_{imp})[2] for CHO cell supernatant[3,]	15 mL supernatant/mL resin[4]
Max flow rate (20 cm bed height, 5 bar)	600 cm/h
pH stability	2 - 13

[1]The average particle size of the cumulative volume distribution
[2]Tested in a 7 × 28 mm column (GoBio™ Mini 1 mL) at 43 cm/h (4 minutes residence time)
[3]CHO cell supernatant contains nucleic acids, proteins, overexpressed mAbs and a diverse range of additional cell components
[4]The DBC_{imp} is based on an impurity assay described in this paper for one specific CHO cell supernatant (used in this study). This value may differ depending on the cell line and expression conditions used.

When WorkBeads 40 TREN is applied upstream WorkBeads affimAb in the process it is used in flow-through mode to adsorb undesirable impurities while allowing mAbs to pass through the column without binding, since the majority of mAbs are basic, and mainly positively charged at neutral or low pH. The heavy sample load of impurities on the WorkBeads affimAb column will thereby be reduced and the purity of the eluted mAb increased, since host cell

nucleic acids and a major portion of the host cell proteins will be adsorbed on WorkBeads 40 TREN.

Experiments

CHO cell supernatant with overexpressed mAbs was loaded onto a WorkBeads affimAb column, see Figure 1. The UV trace shows a large amount of impurities in the flow through (Figure 1, see also highlighted area in Figure 4).

To study the positive effects of WorkBeads 40 TREN as a pretreatment column, a prepacked GoBio Mini™ TREN 5 mL column was placed upstream of the WorkBeads affimAb column. The GoBio Mini TREN column was positioned in a separate valve on the chromatographic system to allow automatic inline or offline flow upstream of the WorkBeads affimAb column. This allows for placing of both columns inline during sample loading and bypass of the GoBio Mini TREN column during mAb elution, and finally also bypass of the WorkBeads affimAb column during regeneration of the GoBio Mini TREN column. Figure 2 shows the hardware setup.

Sample:	20 mL clarified CHO cell supernatant
Column:	WorkBeads affimAb, 6.6 x 100 mm
Binding buffer:	50 mM sodium phosphate, pH 7.4
Elution buffer:	100 mM glycine-HCl, pH 2.7
Flow rate:	1.7 mL/min (equilibration) 0.6 mL/min (sample load) 0.9 mL/min (elution)
Gradient:	Step, 0-100% elution buffer, 10 column volumes (CV)
System:	ÄKTA™ system (GE Healthcare)

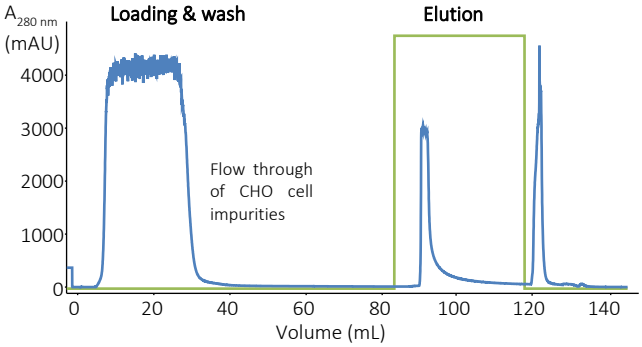

Figure 1. A purification run with only WorkBeads affimAb to demonstrate purification from crude sample by loading a CHO cell supernatant with overexpressed mAbs. Abs280 (blue line) and concentration (%) of elution buffer (green line) are shown in the chromatogram. Injection of sample occurs at time point 0. Cleaning-in-place (CIP) with 0.5 M NaOH occurs after the elution step to regenerate the resin, followed by a re-equilibration step.

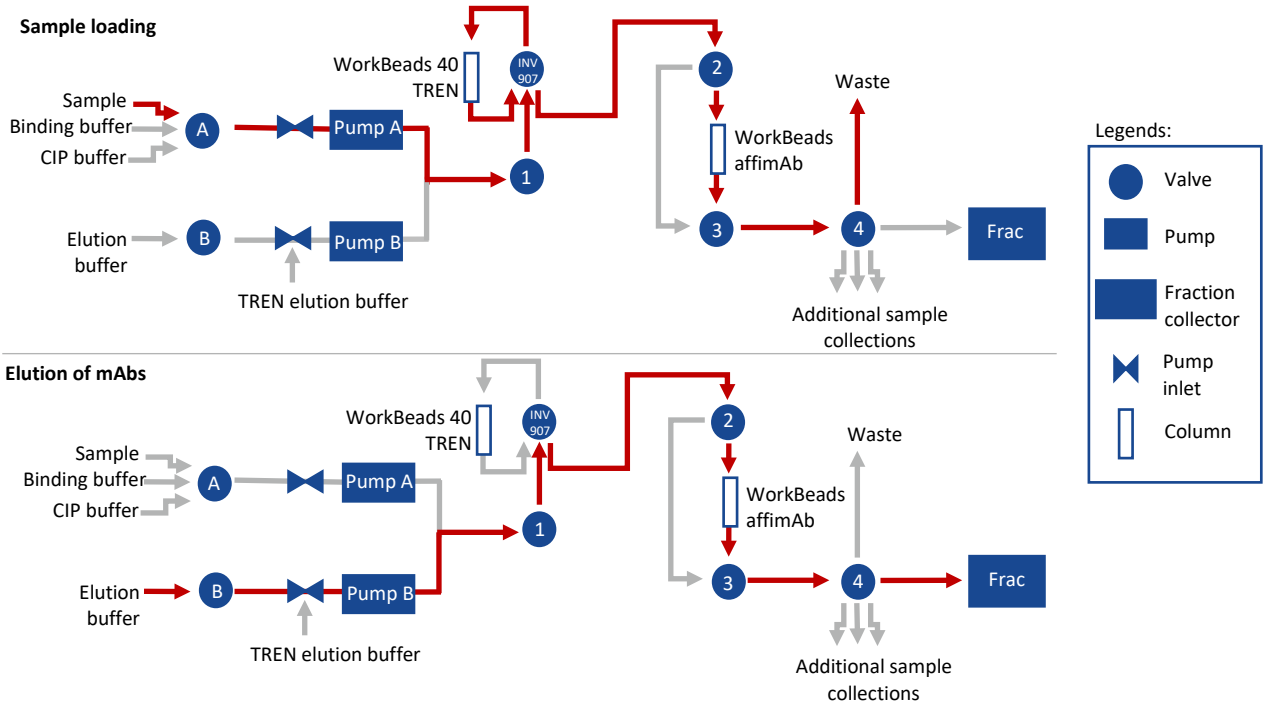

Figure 2. Flow chart of the purification method in which WorkBeads 40 TREN is acting as a pretreatment resin before WorkBeads affimAb. The GoBio Mini TREN column is positioned in Valve INV-907, position 2, and WorkBeads affimAb is positioned between valves 2 and 3. The top flow chart illustrates the flow during sample loading (red arrows) and the bottom flow chart illustrates the flow during the elution of mAbs (red arrows). Other steps in the process are not shown.

In Figure 3 the mAb purification profile is shown at the same sample load and under the same experimental conditions as described in Figure 1. The mAbs were eluted in a single peak and a major part of sample impurities was eluted from WorkBeads 40 TREN in the regeneration steps, which consist of a salt elution step followed by cleaning-in-place (CIP) with 1 M NaOH.

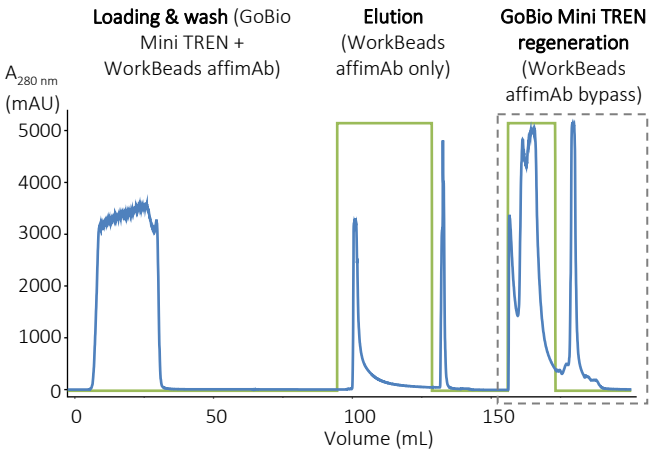

Figure 3. A mAb purification where 20 mL clarified CHO cell supernatant was loaded onto WorkBeads 40 TREN + WorkBeads affimAb connected in series. Impurities in the sample will bind to WorkBeads 40 TREN. Sample loading and washing steps were performed with WorkBeads 40 TREN in-line, but during the elution and CIP steps WorkBeads 40 TREN was bypassed. Regeneration steps were run with the WorkBeads affimAb column in bypass position. Abs280 (blue line), concentration (%) of elution buffer (green line) and regeneration of WorkBeads 40 TREN (dotted grey box) are shown in the chromatogram. Injection of sample occurs at time point 0.

Experimental conditions

To validate the advantage of WorkBeads 40 TREN as a pretreatment resin, a full experiment setup was designed including two protein A resins, WorkBeads affimAb and MabSelect™ SuRe (GE Healthcare), with two different sample loading conditions (low and high sample load). MabSelect SuRe resin was included in our study to compare the performance of WorkBeads affimAb with a market leading protein A resin. Thus, in total eight mAb purifications were performed, where 20 mL or 100 mL clarified CHO cell supernatant were loaded onto the two protein A columns with and without WorkBeads 40 TREN as a pretreatment resin.

In process scale a high percentage of the dynamic binding capacity (DBC) of the resin is loaded to maximize each purification run. Here, the low sample load corresponds to 15% of the DBC for WorkBeads affimAb, whereas the high sample load corresponds to 70% of the DBC for WorkBeads affimAb.

SDS-PAGE analysis of the purifications showed a significant reduction of host cell protein impurities in the mAb eluates when WorkBeads 40 TREN was included upstream. A major portion of HCP was adsorbed to WorkBeads 40 TREN and thus removed from the sample before it was loaded onto the WorkBeads affimAb column (see flow through lanes +/- WorkBeads 40 TREN in Figure 4). The same pattern was seen for the two protein A resins at both low and high sample loads.

These analyses also demonstrated the non-binding of mAbs on WorkBeads 40 TREN at pH 7.4 resulting in no loss of recovery (see mAb elute fractions +/- WorkBeads 40 TREN in Figure 4).

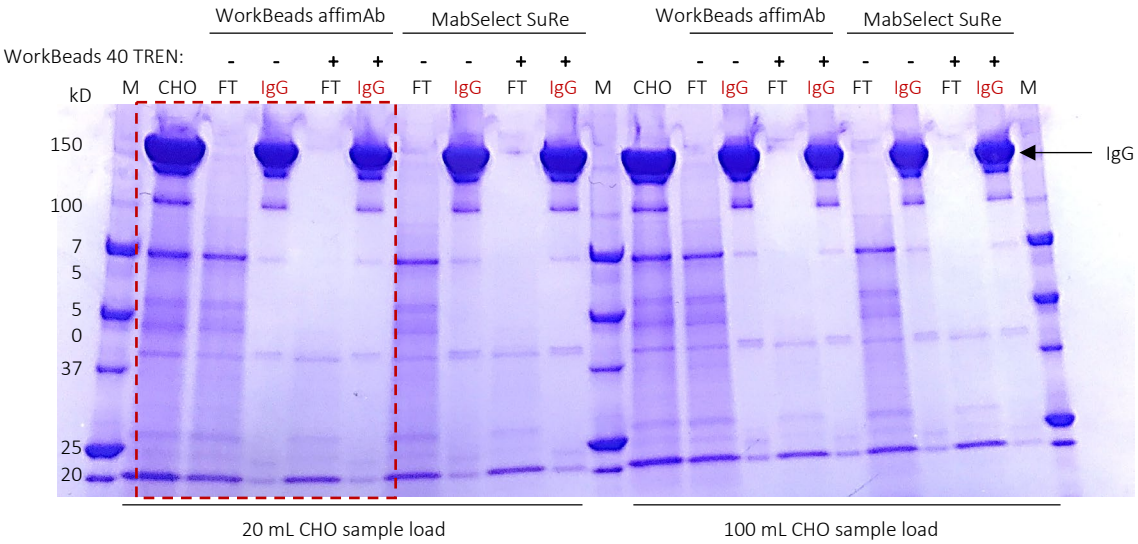

Figure 4. SDS-PAGE analyses of the flow through fractions and the collected mAb eluates. M: marker, CHO: clarified CHO cell supernatant, FT: flow through, IgG: eluted mAb. The highlighted area (red dotted box) illustrates the HCP removal by WorkBeads 40 TREN resin.

The HCP and HCD results of the mAb purifications are shown below in Table 3. The values are presented in ppm levels (ng HCP or ng HCP/mg eluted IgG) since impurities have been shown to mainly coelute with the monoclonal antibodies (this is especially true for agarose-based beads).

Table 3. Analyses of eluates collected from eight combination runs on WorkBeads affimAb and MabSelect SuRe with or without WorkBeads 40 TREN

	Resins	Sample load (mL)	HCP[1*] content (ppm)	HCD[2*] content (ppm)
1.	WorkBeads affimAb	20	667	4
2.	WorkBeads 40 TREN + WorkBeads affimAb	20	224	1
3.	MabSelect SuRe	20	8016	114
4.	WorkBeads 40 TREN + MabSelect SuRe	20	347	1
5.	WorkBeads affimAb	100	2009	12
6.	WorkBeads 40 TREN + WorkBeads affimAb	100	1404	10
7.	MabSelect SuRe	100	10938	80
8.	WorkBeads 40 TREN + MabSelect SuRe	100	7331	69

[1] CHO cell-protein ELISA analysis.

[2] PicoGreen DNA analysis.

[*] The values obtained in this application is based on the experimental conditions and setup as described above. Different sample batches under different conditions will generate variation in absolute numbers obtained.

Low levels of HCP are one of the key quality attributes during downstream process purification development for biopharmaceuticals. HCP analysis using an enzyme-linked immunosorbent assay (ELISA) for measurement of CHO host cell proteins (see Table 3, third column and Figure 5) showed a low level of HCP in the mAb eluates from WorkBeads affimAb at a low sample load.

This value was further reduced with WorkBeads 40 TREN employed upstream of WorkBeads affimAb. The eluted mAbs from MabSelect SuRe contained more than 10-fold higher levels of HCP (~8000 ppm) compared to WorkBeads affimAb eluates (~670 ppm). When WorkBeads 40 TREN was added upstream of MabSelect SuRe, the HCP level was reduced to a 10-fold lower level, indicating its ability to remove protein impurities. At the high sample load (100 mL CHO cell supernatant) the same trend was seen with a 5-fold difference between MabSelect SuRe eluates and WorkBeads affimAb eluates. The absolute HCP levels were increased for all samples, with 1400-2000 ppm for mAb eluates using WorkBeads affimAb and 7300-11000 ppm for mAb eluates using MabSelect SuRe. By including an upstream WorkBeads 40 TREN, all mAb eluates contained a reduced HCP level, but the reductions were more significant at lower sample loads.

An HCP analysis of the flow through fractions of the sample with and without WorkBeads TREN demonstrated an HCP removal of 95%, resulting in a significant reduction of the bioburden on the protein A resins (Figure 5).

Any remaining HCP impurities and tentative mAb aggregates can be removed by applying downstream ion exchange polishing steps to reduce the HCP level below the recommended pharmaceutical requirements.

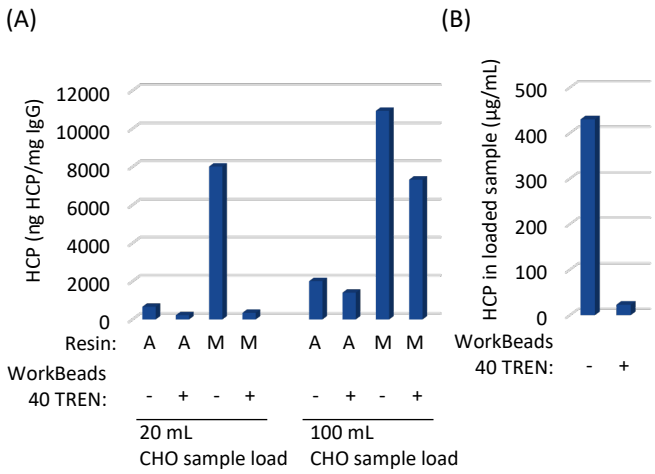

Figure 5. (A) HCP analyses of the collected mAb eluates. A: WorkBeads affimAb, M: MabSelect SuRe. The values are presented in ppm levels (ng HCP or ng HCP/mg eluted IgG). (B) HCP level measurements of flow through CHO cell supernatant, with and without WorkBeads 40 TREN placed upstream.

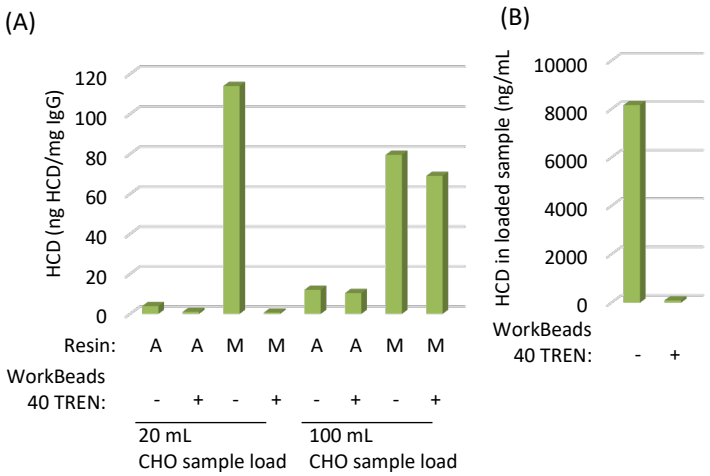

Figure 6. (A) HCD analyses of the collected mAb eluates. A: WorkBeads affimAb, M: MabSelect SuRe. The values are presented in ppm levels (ng HCP or ng HCP/mg eluted IgG). (B) HCD level measurements of flow-through of CHO cell supernatant, with and without WorkBeads 40 TREN.

The HCD (host cell DNA) analysis using the Pico Green assay also showed superior results for WorkBeads affimAb eluates compared to eluates from MabSelect SuRe, with a more than a 20-fold difference, 3.9 ppm *vs* 114 ppm at low sample load (see Table 3, fourth column, and Figure 6). However, adding an upstream WorkBeads 40 TREN column decreased the DNA content to approximately 1 ppm for both eluates, demonstrating the ability of WorkBeads 40 TREN to remove host cell DNA.

At high sample load the absolute HCD levels were higher (~10 ppm for WorkBeads affimAb eluates) and the same trend was observed as for the HCP impurities, *i.e.* addition of a WorkBeads 40 TREN purification step reduced the HCD levels. There were approximately 5-fold higher HCD impurity levels in the eluates from MabSelect SuRe. Analyses of the different flow-through fractions showed that about 99% of the HCD was removed from the sample when a WorkBeads 40 TREN column was connected upstream of the protein A column.

Determination of dynamic binding capacity

To obtain an estimate of the capacity of WorkBeads 40 TREN, a dynamic binding capacity measurement for impurities (DBC_{imp}) was developed. The DBC_{imp} for the specific CHO cell supernatant used in this study was determined by frontal analysis at 10% breakthrough of the impurities at different flow rates/residence times in a GoBio Mini TREN 1 mL column. The level of HCD was used as an indicator of breakthrough since there seems to be a clear correlation between HCP and HCD impurities bound to the resin.

A DBC_{imp} of 15 mL CHO cell supernatant was obtained at 4 minutes residence time in a GoBio Mini TREN 1 mL column. DBC_{imp} correlated with residence times, as expected (see Figure 7). DBC_{imp} values may differ depending on the cell line and expression conditions used.

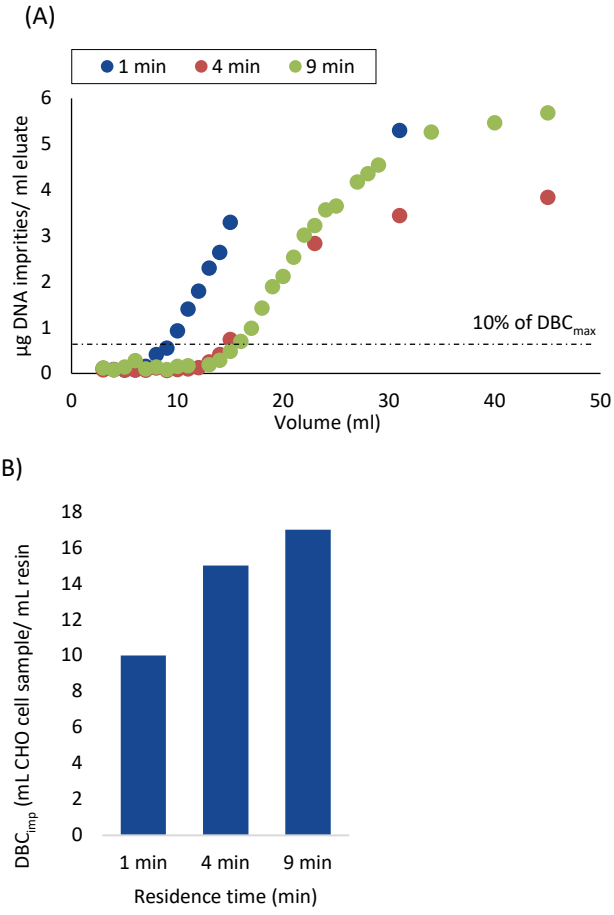

Figure 7. (A) Frontal analysis determination of dynamic binding capacity. (B) Measured DBC_{imp} at a residence time of 1, 4 and 9 minutes in a 7 x 28-mm column (GoBio Mini TREN 1 mL).

Conclusion

WorkBeads 40 TREN is a new and important tool for improving process purification of monoclonal antibodies. Clarified cell supernatants can easily be run through the resin to remove a major portion of impurities such as host cell nucleic acids (DNA and RNA) and host cell proteins. Early removal of these impurities eliminates bioburden on the protein A resin and should thus extend its lifetime. Reduction of impurities early in the purification process further enhances the final purity of the product. This is essential for pharma-ceuticals and diagnostic mAb-based products.

In this study, WorkBeads 40 TREN was used for pretreatment to protect the downstream protein A resin. This resulted in 99% removal of host cell DNA and 95% removal of host cell proteins in the sample load, and additionally also resulted in higher purity of the mAb eluates. WorkBeads 40 TREN can also be placed downstream of the protein A resin as a polishing column in flow-through mode to collect impurities that coelute with the mAb from the protein A column at low pH. This scheme will however not protect the protein A resin.

Due to its orthogonal functionality, regardless of where in the mAb purification process WorkBeads 40 TREN is used, it will significantly improve the purity of the final mAb.

We have also showed that WorkBeads affimAb is a high-performance protein A resin that enables the achievement of extremely high purity of mAbs with respect to both HCD and HCP impurities when compared to the market leading resin MabSelect SuRe.

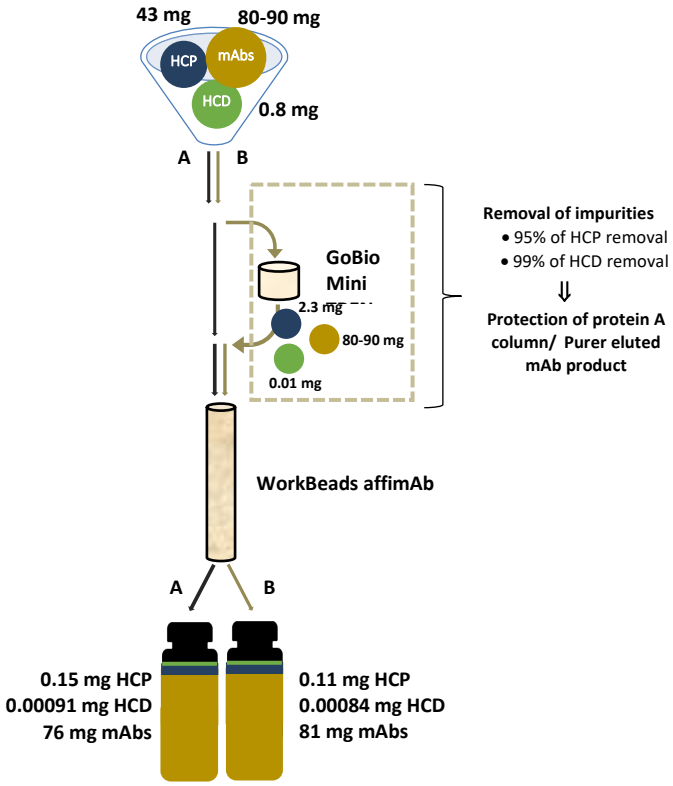

Figure 8. An illustration of reduction of impurities by using WorkBeads 40 TREN for pre-treatment upstream of the WorkBeads affimAb column at a heavy sample load. (A) Direct load of the CHO sample onto the protein A column, (B) Loading via pretreatment. Yellow: mAbs, blue: HCP, and green: HCD.

Order information

Product name	Pack size	Article number
WorkBeads 40 TREN	25 mL	40 603 001
	150 mL	40 603 003
	1 L	40 603 010
	5 L	40 603 050
	10 L	40 603 060
WorkBeads affimAb	25 mL	40 800 001
	200 mL	40 800 003
	1 L	40 800 010
GoBio Mini TREN 1 mL	1 mL x 5	45 655 213
GoBio Mini TREN 5 mL	5 mL x 5	45 655 217
GoBio Mini affimAb 1 mL	1 mL x 5	45 800 103
GoBio Mini affimAb 5 mL	5 mL x 5	45 800 107

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

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

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