Bio-Works

TECHNICAL NOTE

Protection of protein A resin during mAb purification

Purification of monoclonal antibodies (mAbs) is usually carried out using protein A affinity chromatography and results in mAbs with high purity and yield. It is therefore a key step in most mAb purification processes. The mAb-containing feed is often loaded directly onto the protein A column, *e.g.*, WorkBeads™ affimAb, without pretreatment. This causes heavy bioburden on the protein A resin reducing its lifetime. **WorkBeads 40 TREN** is an excellent resin to add as a pretreatment step before the protein A resin in order to reduce the impurities, such as chromatins, host cell DNA (HCD) and other host cell proteins (HCP) that constitute the worst threat for long usage of the protein A resin.

Hints and tips

Sample preparation

Always clarify sample to prevent clogging of the column

Recommendation:	Centrifugation at 10 000 - 20 000 × g for 15–30 minutes
Optional:	Pass though a 0.22 µm–0.45 µm filter
Preferably:	The feed should preferably have the same pH as the binding buffer.

Running conditions

The flow rate of the set-up when using WorkBeads 40 TREN and WorkBeads affimAb in series will depend on:

- Max recommended flow rate of the two different resins
- Column dimensions and hardware
- Residence time
- Capacity requirements

Regeneration of WorkBeads 40 TREN

Impurities from the feed will gradually build-up in the resin over time and reduce the performance of the column. Regular cleaning (Cleaning-in-place, CIP) keeps the resin functional.

Elution buffer:	50 mM $NaPO_4$, 1 M NaCl, pH 7.4, 10 column volumes (CV)
Cleaning-in-place:	0.5–1 M NaOH, 10 minutes contact time
Removal of pigments:	Cycles of 150 mM H_3PO_4 and 1 M NaOH with 5–10 minutes contact time each

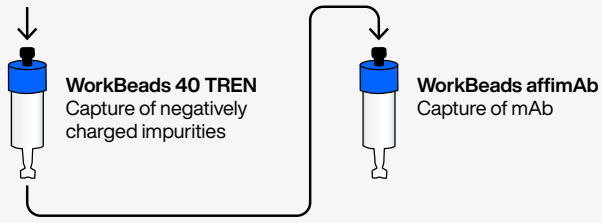
Note: H_3PO_4 may reduce the capacity of the resin after extensive use.
We do NOT recommend storing the resin at low pH.

Note: The coloration of the resin itself may not affect the capacity.

WorkBeads 40 TREN capacity depends on

- mAb concentration in sample
- Running conditions
- Buffer composition
- Flow rate

Note: The WorkBeads 40 TREN resin volume ratio to feed load will differ depending on the feed composition.



Example of running conditions using WorkBeads 40 TREN and WorkBeads affimAb in series for mAb purification.

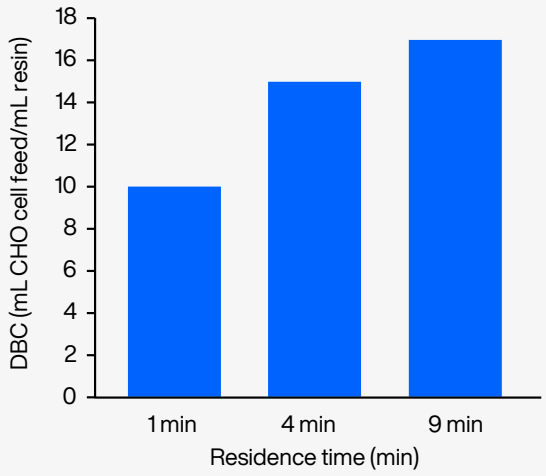
Purification phase	GoBio™ Mini TREN 5 mL	WorkBeads affimAb 6.6 × 100 mm	Buffer
Equilibration *In-line*	75 cm/h 1.7 mL/min	300 cm/h 1.7 mL/min	50 mM $NaPO_4$, pH 7.4 10 CV
Feed loading *Columns connected*	25 cm/h 0.6 mL/min 9 min residence time	100 cm/h 0.6 mL/min 6 min residence time	–
Wash	Disconnected	300 cm/h 1.7 mL/min	50 mM $NaPO_4$, pH 7.4 20 CV
Elution *mAb*	Disconnected	150 cm/h 0.9 mL/min	100 mM glycine-HCl, pH 2.7 5 CV
Elution *impurities*	160 cm/h 3.5 mL/min	Disconnected 10 CV	50 mM $NaPO_4$, 1 M NaCl, pH 7.4
CIP	75 cm/h 1.7 mL/min	Disconnected	0.5–1 M NaOH 10 minutes contact time

Optimizing WorkBeads 40 TREN volume depending on feed composition

The amount of feed that can be applied to the column without saturation depends on the feed composition. A general recommendation for CHO cell supernatant is maximum 10 mL feed per ml WorkBeads 40 TREN. Lower flow rate may allow slightly higher loads. A typical residence time is 3-4 minutes. The maximum load can be determined by analyzing amounts of HCD and HCP in the flow through fractions when applying a feed larger than the expected capacity.

For more detailed information, such as linear flow calculations, see documents:

AN 40 603 001, DS 40 600 020, IN 40 600 020 available on **www.bio-works.com**



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