WorkBeads affimAb

NOTE!
This is a shorter instruction for fast start-up of your purification using WorkBeads™ affimAb.
For the detailed instructions, please, visit www.bio-works.com/documents/

WorkBeads affimAb resin is an alkaline-stable resin designed for purification of monoclonal- and polyclonal antibodies in laboratory to process scale. This resin has a superior basematrix in combination with an optimized alkaline-stable protein A ligand. This results in high dynamic binding capacity also at short residence times, and stabile capacity over multiple purification cycles when doing cleaning-in-place with 0.5 M NaOH. Prepacked BabyBio affimAb 1 ml and 5 ml columns are available for small scale purifications and condition screening in process development.

Column packing

Follow both this general advice when packing a column and the column manufacturer's specific instructions. Preferably, use a column with an adjustable adaptor. In some instances, a packing reservoir or column extension may be needed.
Note: Always make sure that the maximum pressure of the column hardware is not exceeded. Backpressure caused by the chromatography system components connected downstream of the column may reduce the maximum flow that can be used. Wear eye protection.

1. Wash the resin and make a slurry

The resin is provided in 20% ethanol. To avoid undue backpressure when packing, wash the desired amount of resin with several column volumes of deionized water before packing. Add deionized water to the washed resin to obtain a 40% to 60% slurry concentration. Make approximately 15% extra slurry to compensate for the compression during packing. The amount of slurry to be prepared can be calculated according to:

$$\text{Slurry volume} = \frac{\text{bed volume x } 100}{\text{\% slurry}} \text{ x } 1.15$$

2. Pour the slurry into the column

Pour the slurry slowly down the side of the column to avoid formation of air bubbles. Preferably, use a packing reservoir or an extra column tube to extend the column volume to accommodate the entire slurry volume during packing.

3. Pack the bed

Pack the resin with a downward flow higher than the intended operational flow. We recommend 300 cm/h for columns up to 25 mm i.d. and with 200 mm bed height. Make sure the packing flow rate does not exceed the maximum pressure of the column hardware or the resin. The operational flow should not be more than 75% of the packing flow rate.
When the bed height is constant mark the bed height on the column. Stop the flow and remove the packing reservoir or extra tube. Note that the bed height will increase temporarily when the flow is stopped. If needed, adjust the bed height by removing excess resin. Be careful not to remove too much resin. Gently fill the column with packing solution to its rim without disrupting the packed bed. Insert the adjustable adapter on top of the packed bed. Apply a small axial compression of less than 2% of the final bed height by lowering the adapter into the packed bed.

4. Apply a flow

Apply a flow of up to 225 cm/h (taking account of section 3) and check for any gap formation above the surface of the resin bed. If a gap is observed, stop the flow and adjust the adaptor to eliminate the gap.

Purification

The following brief instructions gives general conditions for purification using a column packed with WorkBeads Protein A. Before starting a purification run, it is recommended to make a blank run (with no sample applied) to remove any loosely bound ligands or impurities on the resin. Buffer composition starting point:
Binding buffer: PBS; 20 mM Na-phosphate, 150 mM NaCl, pH 7.4
Elution buffer: 100 mM Na-citrate, pH 3.0
1. Equilibrate the column using 10 column volumes (CV) binding buffer.
2. Apply a clarified sample under neutral conditions.

3. Wash using 10-20 CV binding buffer.
4. Elute with 5 CV elution buffer. Include 100 µl 1 M Tris-HCl, pH 9 per 1 ml collected fraction, to prevent degradation of eluted target protein.
5. Re-equilibrate with 10 CV binding buffer.
6. Equilibrate with 10 CV 20% ethanol for storage.

Sample preparation

Clarify the sample by centrifugation at 10 000 - 20 000 × *g* for 15 - 30 minutes. It is recommended to also pass the sample through a 0.22 - 0.45 µm filter to remove any remaining particles. If the sample contains only small amounts of particles, it may be enough to only carry out filtration. Make sure that the sample has a pH between 5 and 8. Preferably, the sample should have the same pH and ionic strength as the binding buffer.

Optimization, scale-up, additional purification steps, regeneration and CIP

Please, see the detailed instructions, www.bio-works.com/documents/

Storage

Store the resin at 2 to 8°C in 20% ethanol.

Product description

	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
Max recommended flow rate[3]	300 cm/h
Chemical stability	Compatible with all standard aqueous buffers used for protein purification, 10 mM HCl (pH 2), 0.5 M NaOH (pH 12), 0.1 M sodium citrate buffer (pH 3), 6 M guanidine-HCl, 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 (245 cm/h, 2.5 minutes residence time) in a column packed with WorkBeads affimAb resin, column bed 6.6 x 100 mm.
3. Max recommended flow rate at 20 °C using aqueous buffers. Decrease the max flow rate if the liquid has a higher viscosity. Higher viscosities can be caused by low temperature (use half of the max flow rate when operating at 4 °C), or by additives (e.g., use half of the max flow rate for 20% ethanol).

Intended use

WorkBeads affimAb resin is intended for research and industrial purification. The resin shall not be used for preparation of material for clinical or diagnostic purposes.

Safety

Please read the associated Safety Data Sheet (SDS) for WorkBeads affimAb resin, and the safety instructions for any equipment to be used.

Ordering information

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

Bio-Works, Virdings allé 18, SE-754 50 Uppsala, Sweden

Bio-Works