MabCapture™ C High Capacity Protein A Resin, Alkaline Stable

Catalog Number A53031, A53033, A53034

Pub. No. MAN0025916 Rev. A.0

WARNING! Read the Safety Data Sheets (SDSs) and follow the handling instructions. Wear appropriate protective eyewear, clothing, and gloves. Safety Data Sheets (SDSs) are available from thermofisher.com/support.

Product description

The MabCapture™ C High Capacity Protein A Resin, Alkaline Stable is a high binding capacity, alkaline stable, recombinant Protein A–based affinity resin. Recombinant Protein A (rProtein A) has functional activities associated with the C domain of Protein A in that it binds to the CH2-CH3 interface within the Fc region of IgG and shows cross-binding to a subpopulation of VH3 domains. This makes rProtein A suitable for the purification of monoclonal antibodies and a subpopulation of scFvs and Fab fragments that contain a VH3 domain. The ligand is immobilized on a rigid and uniformly-sized agarose bead with an average particle size of 75 µm. The uniform particle size delivers superior performance characteristics over traditional resins.

Product specifications

Table 1 MabCapture™ C High Capacity Protein A Resin, Alkaline Stable product characteristics

Characteristic	Decription
Polymer structure	Cross-linked agarose
Appearance	Spherical, uniformly-sized bead
Functional group	Recombinant, modified Protein A
Dynamic binding capacity	58 mg/mL at 5 minutes residence (80 mg/mL at 10 minutes residence) – 10% breakthrough
pH tolerance	2 to 14
Reusable	Over 100 uses

Contents and storage

Table 2 MabCapture™ C High Capacity Protein A Resin, Alkaline Stable

Cat. No.	Amount[1]	Storage
A53031	10 mL	Store at 4ºC[2]
A53033	50 mL	
A53034	200 mL	

[1] This product is provided as a 50% slurry in 20% ethanol.
[2] Product is shipped at ambient temperature.

Required materials not supplied

Unless otherwise indicated, all materials are available through **thermofisher.com**. "MLS" indicates that the material is available from **fisherscientific.com** or another major laboratory supplier. Catalog numbers that appear as links open the web pages for those products.

Product	Cat. No.
Reagents	
Equilibration and binding buffer, one of the following, or equivalent: • Pierce™ 20X Phosphate Buffered Saline • BupH™ Phosphate Buffered Saline Packs • Pierce™ 20X TBS Buffer • BupH™ Tris Buffered Saline Packs	 • 28348 • 28372 • 28358 • 28376
Elution buffer: • Pierce™ IgG Elution Buffer, pH 2.0 • 0.1 M sodium citrate, pH 3.0 • 0.1 M glycine, pH 2.0	 • 21028 • MLS • MLS
For resin regeneration: • 0.1 M glycine, pH 2.0 (required if using 0.1 M sodium citrate for elution) • 0.2 M sodium hydroxide (NaOH), pH 13	MLS
Instrument and Equipment	
Liquid chromatography (LC) or Fast protein liquid chromatography (FPLC) system	MLS
Empty column for resin packing [1]	

[1] Follow the manufacturer instructions to pack column.

Procedural guidelines

- Protein yield and purity are dependent upon the expression level, conformation, and solubility characteristics of the protein of interest. Therefore, it is important to optimize these parameters before attempting a large-scale purification.
- For optimal recovery, use a sample size with an expected IgG load that is <80% of the binding capacity of the column. The concentration of antibody in tissue culture supernatant varies considerably among hybridoma clones.
- For liquid chromatography applications, use highly pure buffer components and ultrapure water. Degas or filter buffers through a 0.45 µm filter before use.
- The MabCapture™ C High Capacity Protein A Resin, Alkaline Stable may be used over 100 times without affecting protein yield or purity (depending on sample type). Between each use, regenerate the resin as described to remove residual or nonspecifically adsorbed protein (see “Regenerate MabCapture™ C High Capacity Protein A Resin, Alkaline Stable” on page 4).
- The MabCapture™ C High Capacity Protein A Resin, Alkaline Stable allows for purification strategy customization. Purification conditions can be scaled as needed. The procedure may be performed at room temperature or at 4ºC.
- Monitor protein elution by measuring the absorbance of the fractions at 280 nm. The eluted protein can be directly analyzed by SDS-PAGE.

Purify proteins by gravity

1. Mix the bottle of resin by inversion until the suspension is homogeneous, then transfer 2 mL of the 50% suspension (corresponding to 1 mL bed volume) to a 15–mL conical tube. Allow the resin to settle by gravity, then remove the supernatant.

   Note: Alternatively, resin equilibration can be performed directly in a disposable gravity flow column (see **thermofisher.com** for Pierce™ disposable columns).

2. Add 10 mL of equilibration buffer, then gently resuspend the slurry to equilibrate the resin. Allow the resin to settle by gravity, then remove 10 mL of supernatant.

3. Add 10 mL of clarified sample to the equilibrated resin, then incubate at 4ºC for 1 hour on an end-over-end shaker.

   Note: Alternatively, batch binding can be performed directly in a gravity flow column with closed bottom and top outlets.

4. Transfer the binding suspension to a disposable gravity flow column with a capped bottom outlet. Rinse the centrifuge tube with equilibration buffer to remove any resin that is adhered to the wall.
5. Remove the bottom cap of the column, then collect the flow-through fraction.
6. Wash the column 3 times with 5 mL of equilibration buffer.
7. Elute the protein with elution buffer. Collect 5 × 0.5 mL fractions, using a separate tube for each eluate.

   (*Optional*) To increase protein yields, incubate the resin for 15 minutes in elution buffer before collecting the eluate.

Regenerate the resin for immediate reuse or storage (see "Regenerate MabCapture™ C High Capacity Protein A Resin, Alkaline Stable" on page 4).

Purify proteins by centrifuge column

1. Mix the bottle of resin by inversion until the suspension is homogeneous, then transfer 100 µL of the 50% suspension (corresponding to 50 µL bed volume) to a 0.8 mL centrifuge column (Cat. No. 89868).
2. Place the column in a 1.5-mL centrifuge tube, then centrifuge at 1,000 × *g* for 2 minutes. Discard the flow-through.
3. Add 0.5 mL of equilibration buffer, then gently resuspend the slurry to equilibrate the resin.
4. Centrifuge the column at 1,000 × *g* for 2 minutes. Discard the flow-through.
5. Add 0.5 mL of clarified sample to the spin column containing the equilibrated resin, then cap and plug the column. Incubate the spin column at 4ºC for 1 hour on an end-over-end shaker.
6. Transfer the centrifuge column to a new 1.5–mL centrifuge tube, then remove the cap and bottom plug.
7. Centrifuge the column at 1,000 × *g* for 2 minutes to collect the flow-through or unbound fraction.
8. Discard the collection tube, then place the column on a new 1.5-mL centrifuge tube.
9. Wash the column 3 times with 0.5 mL of equilibration buffer, then centrifuge at 1,000 × *g* for 2 minutes to collect each wash volume.
10. Discard the collection tube, then place the column on a new 1.5-mL centrifuge tube.
11. Add 0.1 mL of elution buffer to the column, then centrifuge at 1,000 × *g* for 2 minutes. Repeat 4 times, collecting each eluate in a separate tube.

    (*Optional*) To increase protein yields, incubate the resin for 15 minutes in elution buffer before collecting the eluate.

Regenerate the resin for immediate reuse or storage (see "Regenerate MabCapture™ C High Capacity Protein A Resin, Alkaline Stable" on page 4).

Purify proteins using automated chromatography systems

Purifications can be performed at room temperature or 4ºC. Ensure all solutions are degassed.

For optimal binding capacity, we recommend a flow rate of 150 cm/hour, however, up to 300 cm/hour can be used

1. Pack an appropriately–sized column with resin according to the manufacturer instructions. Ensure the packing flow rate is at least 20% faster than the flow rate that will be used during purification.
2. Equilibrate the column and all buffers to working temperature.
3. Prepare the LC system.
   a. Wash pumps and fill tubing with buffer.
   b. Allow a few drops of buffer to flow from the tubing into the column top to avoid introducing air into the system.
   c. Connect the column to the tubing.
4. Equilibrate the column with 5—10 column volumes (CV) of equilibration buffer at a flow rate of 150 cm/hour.

5. For maximum binding, dilute the sample 1:1 with equilibration buffer to adjust the sample to the ionic strength and pH of the equilibration buffer. If the sample contains insoluble matter, centrifuge or filter (0.45 µm filter) before use.

6. Apply the sample to the column at a flow rate of 150 cm/hour. Ensure the sample volume does not exceed the column binding capacity for target protein.

   Note: Binding capacity is flow rate– and protein–dependent. Decreasing the flow rate during the sample load will increase binding capacity. Higher flow rates will decrease production time but can result in losing a small portion of the target protein in the flow-through fraction.

7. Wash the resin at a flow rate of 150 cm/hour with 10–15 CV of equilibration buffer or until the absorbance at 280 nm approaches baseline.

8. Elute at a flow rate of 150 cm/hour with approximately 5–10 CV of elution buffer and collect fractions.

For regeneration and storage, see "Regenerate MabCapture™ C High Capacity Protein A Resin, Alkaline Stable" on page 4.

Regenerate MabCapture™ C High Capacity Protein A Resin, Alkaline Stable

The MabCapture™ C High Capacity Protein A Resin, Alkaline Stable may be used over 100 times without affecting protein yield or purity (depending on sample type). Perform the following procedure between each use to remove residual or nonspecifically adsorbed protein.

1. Wash resin with 10 column volumes (CV) of equilibration buffer.

2. If 0.1 M sodium citrate, pH 3.0, is used as the elution buffer, wash resin with 10 CV of 0.1 M glycine at pH 2.0. If 0.1 M glycine is used as the elution buffer, skip step 2 and move directly to step 3.

3. Wash resin with 10 CV of 0.2 M NaOH at pH 13.0.

4. Wash resin with 10 CV of equilibration buffer.

Regenerated resin can be used immediately for purification or stored at 4°C as a 50% slurry in 20% ethanol.

Related products

Product	Cat. No.
Expi293™ Expression System Kit	A14635
ExpiCHO™ Expression System Kit	A29133
ExpiSf™ Expression System Starter Kit	A38841
Pierce™ Gentle Ag/Ab Elution Buffer, pH 6.6 500 mL	21027
Pierce™ IgG Elution Buffer, pH 2.0	21028
Pierce™ 20X Phosphate Buffered Saline	28348
Pierce™ 20X TBS Buffer	28358
BupH™ Phosphate Buffered Saline Packs	28372
BupH™ Tris Buffered Saline Packs	28376
Pierce™ Protease and Phosphatase Inhibitor Mini Tablets	A32959
Halt™ Protease Inhibitor Cocktail (100X)	87786
Pierce™ Protein Concentrators, PES	88514, 88516, 88521, 88538, 88523, 88525, 88527, 88529
Novex™ WedgeWell™ 10%, Tris-Glycine, 1.0 mm, Mini Protein Gel, 10-well	XP00100PK2, XP00100BOX
Zeba™ Spin Desalting Columns	89882, 89889, 89891, 89893, 87766, 87768, 87770, 87772
Slide-A-Lyzer™ Dialysis Cassettes	66380, 66810, 66383, 66830, 66003, 66005, 66012, 66030

Troubleshooting

Observation	Possible cause	Recommended action
Low protein yield	There was poor expression of soluble protein.	Optimize expression conditions.
	Antibody of interest was inactivated due to the elution buffer having a low pH.	Use Pierce™ Gentle Ag/Ab Elution Buffer, pH 6.6 (Cat. No. 21027, 21013).
	The sample was devoid of antibody species, or of a subclass that binds to Protein A.	Determine binding characteristics of sample and determine if optimized for use with Protein A.
Poor protein purity	There was insufficient washing during the experiment.	Wash resin additional times, or modify pH of the equilibration buffer or wash buffer.
Slow column flow	The column was overloaded.	Apply less protein extract onto the column, and make sure the extract is not too viscous or highly particulate.

Limited product warranty

Life Technologies Corporation and/or its affiliate(s) warrant their products as set forth in the Life Technologies' General Terms and Conditions of Sale at **www.thermofisher.com/us/en/home/global/terms-and-conditions.html**. If you have any questions, please contact Life Technologies at **www.thermofisher.com/support**.

Thermo Fisher Scientific | 3747 N. Meridian Road | Rockford, Illinois 61101 USA
For descriptions of symbols on product labels or product documents, go to thermofisher.com/symbols-definition.

Revision history: Pub. No. MAN0025916

Revision	Date	Description
A.0	6 December 2021	New document for MabCapture™ C High Capacity Protein A Resin, Alkaline Stable.