CaptureSelect™ Human Albumin Affinity Matrix

Catalog Numbers 1912970250, 1912970500, 19129701L, 19129705L

Pub. No. MAN0009649 **Rev.** C.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 CaptureSelect™ Human Albumin Affinity Matrix purifies human albumin and human albumin fusion proteins from complex source materials (such as cell cultures, plasma, and serum) in a single step.

The matrix combines selectivity for a unique domain of human serum albumin (HSA) with the benefits of a robust and high-quality affinity matrix provided by a 13 kDa heavy chain antibody fragment.

Product advantages

The CaptureSelect™ Human Albumin Affinity Matrix offers:

- High recovery and purity in a single step
- Neutral pH elution conditions to retain the biological activity of human albumin and human albumin fusion proteins; the mild elution conditions make this matrix extremely suitable for purifying albumin fusion proteins that are susceptible to low pH environments
- Compatibility with FPLC systems

Specifications

Ligand	CaptureSelect™ Human Albumin Affinity Ligand
Binding specificity	Human albumin from recombinant sources, plasma, and serum
Matrix and particle size	Aldehyde-activated agarose, 65 µm
Dynamic binding capacity	~12 g of albumin/L of matrix (5% breakthrough at 150 cm/h)
Shipping solution	0.1 M Tris, 1.0 M NaCl, 20% (v/v) ethanol pH 8.0

Conditions for use

Parameter	Conditions for use
Equilibration buffer	20 mM Tris or PBS, pH 7.0–7.5
Elution buffer	• **Neutral:** 20 mM Tris, pH 7.4, with one of the following: – 2 M $MgCl_2$ – 1 M NaCl, 50% (v/v) propylene glycol (PG), pH 7.4 – 1 M NaCl, 0.5 M arginine, pH 7.4 • **Acidic:** 0.1 M glycine, pH 3.0
Strip buffer	Any of the following: • 0.1 M glycine, pH 2.0 • 0.1–1.0 M Acetic acid • 0.1–0.5 M Citric acid
Flow rate	50–200 cm/h
Pressure limit	≤ 2 bar
Cleaning solution	Any of the following: • 6.0 M Guanidine HCl • 10–20 mM NaOH (Higher concentrations affect the functionality of the affinity ligand on the matrix.) • PAB (120 mM phosphoric acid, 167 mM acetic acid, and 2.2% (v/v) benzyl alcohol) (Rogers *et al.*, 2009) Freshly prepare PAB every 4–5 days and store protected from light to minimize radicals that affect the functionality of the matrix.
Storage solution	0.1 M Tris, 1.0 M NaCl, 20% (v/v) ethanol pH 8.0
Operating and storage temperatures	• **Operating**: 2–25°C • **Storage**: 2–8°C

Flow characteristics

You can use agarose-based CaptureSelect™ affinity matrices at flow rates of 50–300 cm/h (Figure 1).

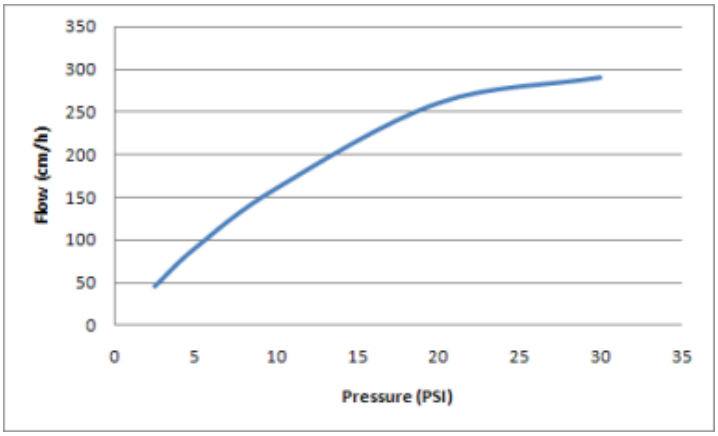

Fig. 1 Pressure-flow properties of an agarose-based CaptureSelect™ matrix tested on a 10-cm diameter column packed to 16-cm bed height. The resin can be operated at flow rates up to 300 cm/h, with a pressure drop that allows use in conventional low-pressure chromatography columns and systems.

However, for optimal binding capacity and elution efficiency, we recommend flow rates of 50–200 cm/h. A low flow rate results in longer contact time of the load with the affinity matrix and drives the binding capacity (Figure 2).

Caution: For manufacturing, processing or repacking.

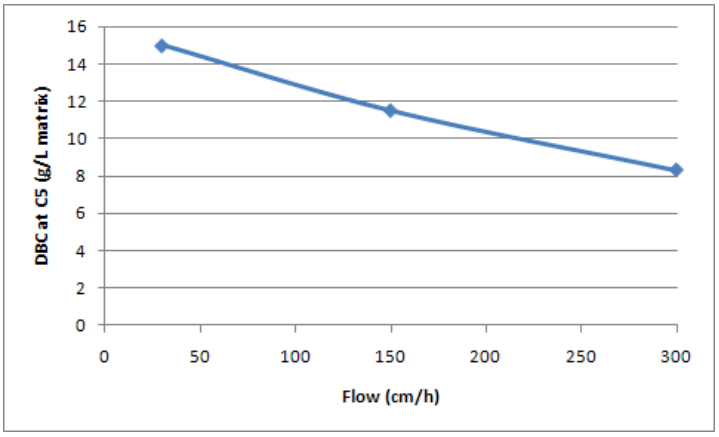

Fig. 2 The dynamic binding capacity of the CaptureSelect™ Human Albumin Affinity Matrix at 5% breakthrough (C5) as a function of the linear flow rate with human albumin as a load on a 5-mm x 20-mm column.

In addition, the elution fraction is more concentrated at a lower flow rate (Figure 3).

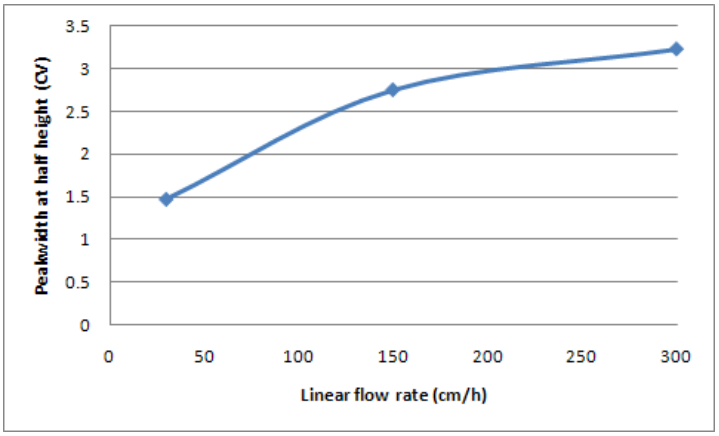

Fig. 3 The peak width at half height (in column volumes) of the elution peak versus the linear flow rate of agarose-based CaptureSelect™ matrices. Lowering the flow rate during elution reduces the volume of the elution peak.

We recommend that you optimize each of your specific processes to achieve the best conditions for process time, binding capacity, and elution efficiency.

Guidelines for use - FPLC

For optimal matrix performance, optimize the conditions in the following procedure for your application.

1. Pack the column as described in *CaptureSelect™ Affinity Matrices: Guidelines for Packing* (Pub. no. MAN0009645).
2. Attach the packed column to the FPLC system.
3. Equilibrate the matrix with 10 column volumes (CVs) of equilibration buffer.
4. Determine the volume of sample to load, based on the dynamic binding capacity, concentration of the target molecule, and the column size. Optimum loading is at physiological pH. Avoid acidic conditions, which decrease binding efficiency.
5. Load the sample on the column.
6. Wash the sample with 5–10 CVs of equilibration buffer. To optimize washing efficiency, you can add NaCl to the equilibration buffer (up to 1.0 M).
7. Elute with 3–5 CVs of elution buffer.
8. Re-equilibrate the column in equilibration buffer.
9. Strip the column with 0.1 M glycine (pH 2.0), citric acid, or acetic acid (0.1–1.0 M).
10. Re-equilibrate the column in equilibration buffer to prepare the column for another purification run.
11. If the column will not be used immediately, store the matrix according to the storage parameters provided in "Conditions for use" on page 1.

Column cleaning guidelines

Resin lifetime depends on how the resin is used and cleaned. Therefore, we recommend that you specifically evaluate each purification process.

Typical cleaning procedures for CaptureSelect™ resins include combinations of acidic stripping followed by low concentrations of NaOH, before storing in 0.1 M Tris, 1.0 M NaCl, 20% (v/v) ethanol pH 8.0 (Eifler *et al.*, 2014). For acidic stripping, citric acid in the range of 0.1–0.5 M is preferred due to its chelating properties. For cleaning, besides NaOH, other cleaning agents that can be used are 6.0 M guanidine HCl or PAB. The CaptureSelect™ Human Albumin Affinity Matrix was exposed to several cleaning agents for up to 96 hours at ambient temperature. The functionality of the resin was measured every 24 hours to test compatibility of the matrix with these cleaning agents (Figure 4).

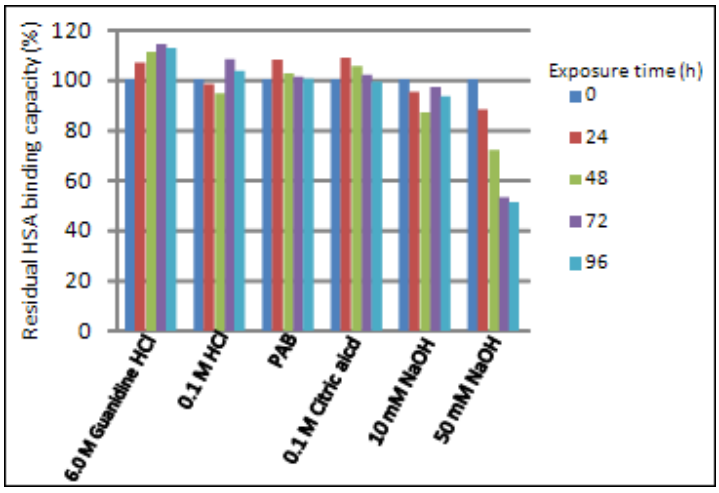

Fig. 4 The CaptureSelect™ Human Albumin Affinity Matrix is compatible with acidic and mildly caustic cleaning agents for up to 96 hours at ambient temperature. In addition, chaotropic agents like guanidine-HCl are compatible with the resin.

To optimize column cleaning, consider these guidelines:

- Pump the cleaning solution through the column for 15–20 minutes in upflow.
- Incorporate a static hold to increase the time that the cleaning solution is in the column while minimizing the volume of cleaning solution required.
- When a combination of acidic and mildly caustic cleaning agents is used, apply the NaOH solution as a final cleaning agent to minimize the risk of irreversibly binding impurities on the column.
- In some purification processes, 20% (v/v) isopropanol (with or without acid) and 6.0 M guanidine HCl can help remove discoloration.

Example application - FPLC

In this example, an albumin fusion protein was purified from clarified cell culture harvest. After the resin was loaded, the column was equilibrated, then eluted. Conditions were as follows:

- **Column** – CaptureSelect™ Human Albumin Affinity Matrix
- **Equilibration buffer** – PBS, pH 7.4
- **Load** – Feedstock of recombinant albumin fusion production

- **Elution buffer** – 20 mM Tris with 2.0 M $MgCl_2$, pH 7.4
- **Flow** – 200 cm/h

The fusion protein elutes well under these conditions. As shown in Figure 5, minimal protein is detected in the pH strip at pH 2.0.

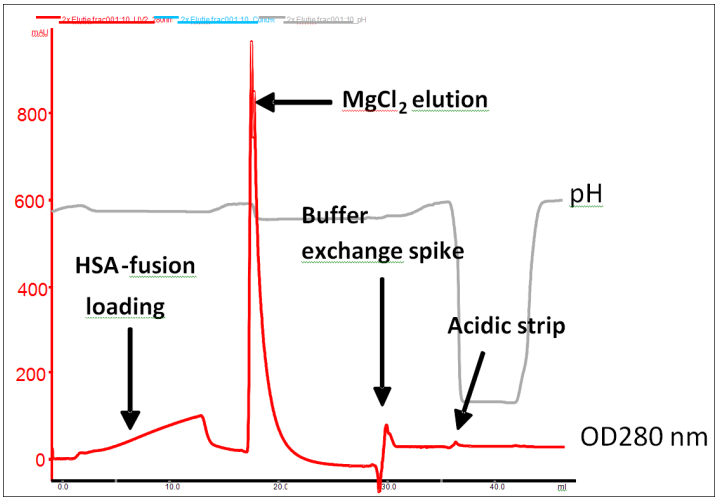

Fig. 5 Chromatogram of an albumin fusion protein purified using the CaptureSelect™ Human Albumin Affinity Matrix.

Red line: OD280 signal; Grey line: pH value

The collected fractions were analyzed on a Coomassie non-reduced SDS-PAGE, showing a highly pure albumin fusion protein in the elution fraction (Figure 6).

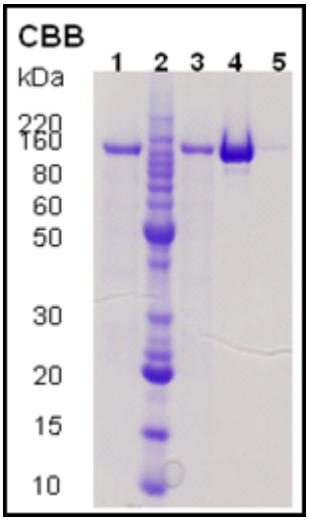

Fig. 6 SDS PAGE analysis of the fractions: Non-reduced Coomassie-stained SDS PAGE (CBB).

Lane 1: starting material; Lane 2 molecular weight marker; Lane 3: flow through; Lanes 4 and 5: elution fractions

Ordering information

Product	Size	Cat. no.
CaptureSelect™ Human Albumin Affinity Matrix	250 mL	1912970250
	500 mL	1912970500
	1 L	19129701L
	5 L	19129705L

Regulatory support

A Regulatory Support File (RSF) is available that contains detailed information about the resin and the manufacturing process. For more information about the RSF, contact your local sales representative.

Supporting products

Pre-packed affinity HPLC columns are available for determining titers and analyzing in-process samples during the production and purification of HSA-fusion proteins. The pre-packed columns contain the same affinity ligand as the CaptureSelect™ Human Albumin Affinity Matrix, but the ligand is immobilized on POROS™ 20-µm beads that are suitable for HPLC applications.

In addition, a ligand leakage ELISA is available for detecting possible leached ligand in the elution fractions of the CaptureSelect™ Human Albumin Affinity Matrix.

Product	Size	Cat. no.
POROS™ CaptureSelect™ HSA Affinity Column	2.1 × 30 mm	4469151
	4.6 × 50 mm	4469165
	4.6 × 100 mm	4469170
	10 × 100 mm	4469175
CaptureSelect™ HSA Ligand Leakage ELISA Kit	1 assay	810297001
	10 assays	810297010

For more information

For more information on CaptureSelect™ products and ligand leakage ELISA products, go to **www.thermofisher.com/captureselect**.

Customer and technical support

Visit **thermofisher.com/support** for the latest in services and support, including:

- Worldwide contact telephone numbers
- Product support, including:
  - Product FAQs
  - Software, patches, and updates
- Order and web support
- Product documentation, including:
  - User guides, manuals, and protocols
  - Certificates of Analysis
  - Safety Data Sheets (SDSs; also known as MSDSs)

  Note: For SDSs for reagents and chemicals from other manufacturers, contact the manufacturer.

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 found on Life Technologies' website 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**.

References

Rogers, M. *et al.* 2009. Development of a rapid sanitization solution for silica-based protein A affinity adsorbents. *Journal of Chromatography A.* 1216:4589–4596.

Eifler, N. *et al.* 2014. Development of a novel affinity chromatography resin for platform purification of lambda fabs. *Biotechnology Progress* DOI:10.1002/btpr.1958.

The information in this guide is subject to change without notice.

DISCLAIMER

TO THE EXTENT ALLOWED BY LAW, LIFE TECHNOLOGIES AND/OR ITS AFFILIATE(S) WILL NOT BE LIABLE FOR SPECIAL, INCIDENTAL, INDIRECT, PUNITIVE, MULTIPLE, OR CONSEQUENTIAL DAMAGES IN CONNECTION WITH OR ARISING FROM THIS DOCUMENT, INCLUDING YOUR USE OF IT.

Table 1 Revision history of Pub. no. MAN0009649

Revision	Date	Description
C.0	08 March 2016	Buffer changes; updated format, legal, and support content.