CaptureSelect™ Factor X Affinity Matrix

Catalog Numbers 1943702250, 194370201L, and 194370205L

Pub. No. MAN0019518 Rev. B.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™ Factor X Affinity Matrix purifies human Factor X from plasma and recombinant sources directly from clarified cell culture harvest in a single step.

The matrix has selectivity for recombinant Factor X by making use of a 14 kDa single domain (VHH) antibody produced in yeast. It is completely free of animal-derived components. The ligand is immobilized on a high-quality chromatography matrix.

The CaptureSelect™ Factor X Affinity Matrix offers:

- High recovery and purity in a single step
- Selective binding of human Factor X and Factor Xa
- Binding in the presence of calcium
- Neutral pH elution with addition of EDTA

Contents and storage

Table 1 CaptureSelect™ Factor X Affinity Matrix

Cat. No.	Amount	Storage
1943702250	250 mL	Room temperature for <2 weeks, long-term storage at 2–8°C.
194370201L	1 L	
194370205L	5 L	

Specifications

Characteristic	Description
Ligand	CaptureSelect™ Factor X Affinity Ligand
Binding specificity	Factor X, from recombinant and plasma sources
Matrix and particle size	Epoxide-activated agarose, 65 µm
Dynamic binding capacity	10 g Factor X/L of matrix
Shipping solution	20% (v/v) ethanol

Conditions for use

Parameter	Conditions for use
Equilibration buffer	50 mM Tris, 0.15 M NaCl, 5 mM $CaCl_2$ pH 7
Elution buffer	50 mM Tris, 150 mM NaCl, 5 mM EDTA, pH 7
Strip buffer	Any of the following: • 0.1 M glycine, pH 2.0 • 0.5 M acetic acid • 0.5 M citric acid
Flow rate	50–200 cm/h
Pressure limit	≤2 bar
Cleaning solution	Any of the following: • 0.5 M acetic acid • 0.5 M citric acid • 10 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 can affect the functionality of the matrix. • 2.0 M Guanidine HCl
Storage solution	20% (v/v) ethanol
Operating temperature	2–25°C

For Research Use or Further Manufacturing. Not for diagnostic use or direct administration in humans or animals.

Flow characteristics

You can use agarose-based CaptureSelect™ affinity matrices at flow rates of 50–250 cm/h (Figure 1).

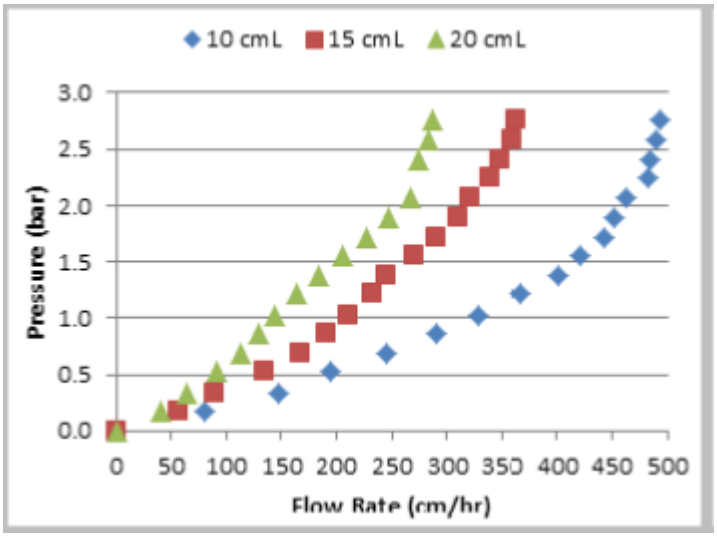

Figure 1 Typical pressure-flow curve for CaptureSelect™ resin at increasing bed heights: 10 cm (blue), 15 cm (red), and 20 cm (green). (10-cm diameter column packed at 3 bar; mobile phase=0.1 M NaCl.)

The resin can be operated at flow rates up to 250 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 50–150 cm/h. A low flow rate results in longer contact time of the load with the affinity matrix and drives the binding capacity. In addition, the elution fraction is more concentrated at a lower flow rate.

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 with chromatography systems

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 chromatography 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 (0.5 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.

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 cleaning followed by low concentrations of NaOH, before storing in 20% (v/v) ethanol at neutral pH (Eifler *et al.*, 2014).

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.

Example application

In this example, plasma derived Factor X was purified from 14 mL human plasma containing 25 IU/mL Factor X, resulting in a high yield, very efficient elution, and high purity. The following conditions were used:

- **Column**—0.4-mL (5 x 20 mm) CaptureSelect™ Factor X
- **Load**—Plasma protein fraction containing 25 IU/mL Factor X mixed 1:1 with 50 mM Tris, 150 mM NaCl, 5 mM $CaCl_2$, pH 7
- **Elution buffer**—50 mM Tris,150 mM NaCl, 5 mM EDTA, pH 7.0
- **Strip**—0.1 M Glycine, pH 2.0

A chromatogram of the purification run (Figure 2) and a SDS PAGE analysis of the starting material, flow through, and elution fractions (Figure 3) detail the purification process.

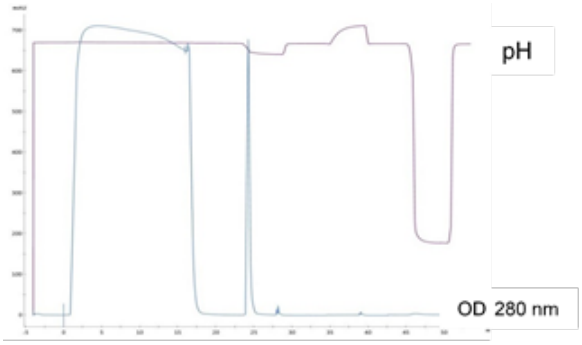

Figure 2 Chromatogram of the purification run

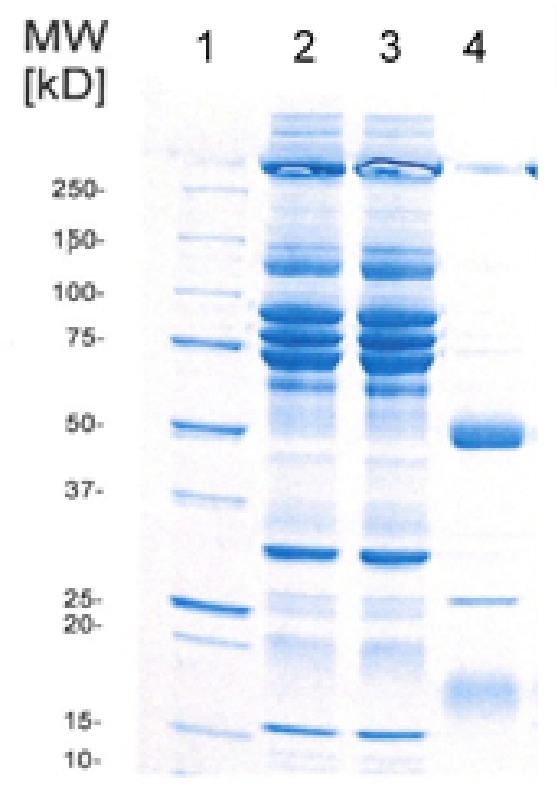

Figure 3 SDS PAGE analysis of the load, flow through, and elution fractions on a reducing SDS-PAGE gel (CBB stained)

① Lane 1: MW marker
② Lane 2: Load
③ Lane 3: Flow through
④ Lane 4: Elution

Factor X is cleaved into the following 3 chains: Factor X heavy chain (42 kDa), Factor X light chain (16 kDa) and Activated Factor Xa heavy chain (29 kDa).

Supporting products

A biotinylated anti-Factor X conjugate is available. Applications for the CaptureSelect™ Biotin Anti-Factor X conjugate include:

- ELISA
- Western blot
- Gyros™ Gyrolab™-based immunoassays
- Label-free detection platforms, such as those based on surface plasmon resonance (Biacore™ and IBIX-MX96 systems) and bio-layer interferometry (Octet™ systems)

A ligand leakage ELISA is available for detecting possible leached ligand in the elution fractions of the CaptureSelect™ Factor X Affinity Matrix.

Product	Size	Cat. no.
CaptureSelect™ Biotin Anti-Factor X conjugate	100 µg	7103702100
	500 µg	7103702500
CaptureSelect™ Factor X Ligand Leakage ELISA	1 assay	810370201
	10 assays	810370210

Regulatory Support File

A Regulatory Support File (RSF) is available for the resin. It contains detailed information about the resin and the manufacturing process. Contact your local sales representative to obtain access.

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 service and support information.

- Worldwide contact telephone numbers
- Product support information
  - Product FAQs
  - Software, patches, and updates
  - Training for many applications and instruments
- Order and web support
- Product documentation
  - 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

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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.

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For descriptions of symbols on product labels or product documents, go to thermofisher.com/symbols-definition.

Revision history: Pub. No. MAN0019518 B.0

Revision	Date	Description
B.0	16 May 2023	• The CaptureSelect™ IgE EvolveD™ Column products were removed. • The regulatory statement was updated.
A.0	23 September 2020	New document for the CaptureSelect™ Factor X Affinity Matrix.

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

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