CaptureSelect™ FcXL Affinity Matrix

Catalog Number 1943280250, 1943280500, 19432801L, 19432805L

Pub. No. MAN0013480 **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 **www.lifetechnologies.com/support**.

Product description

The CaptureSelect™ FcXL Affinity Matrix purifies human IgG and Fc fusion proteins from complex source materials (such as cell culture medium, human plasma, and serum) in a single step. The affinity matrix is human-specific and does not bind IgG from other species (including bovine, horse, or rodents).

The matrix combines selectivity for the CH3 domain of human IgG, recognizing all four subclasses (IgG1, IgG2, IgG3, and IgG4) with the benefits of a robust and high-quality affinity matrix provided by a 13 kDa llama heavy chain antibody fragment.

Product advantages

The CaptureSelect™ FcXL Affinity Matrix offers:

- High recovery and purity in a single step
- Mild pH elution conditions to retain biological activity and prevent aggregation of Fc fusion proteins and IgG
- Compatibility with FPLC systems

Specifications

Ligand	CaptureSelect™ FcXL Affinity Matrix
Binding specificity	Human IgG (all four subclasses) and Fc fusion proteins
Matrix and particle size	Aldehyde-activated agarose, 65 µm
Dynamic binding capacity	25 g of IgG/L of matrix (10% breakthrough at 5 minutes residence time)
Shipping solution	20% (v/v) ethanol

Conditions for use

Parameter	Conditions for use
Equilibration buffer	20 mM Tris or PBS, pH 7.0–7.5
Elution buffer	• **Mild pH:** 20 mM sodium acetate with 1.0 M $MgCl_2$, and 40% (v/v) propylene glycol, pH 5–6 • **Acidic:** 20 mM acetic acid or citric acid, 0.1 M glycine, pH 3–4
Strip buffer	Any of the following: • 0.1 M glycine, pH 2.0 • 0.1–1.0 M acetic acid • Citric acid
Flow rate	50–200 cm/h
Pressure limit	≤ 2 bar
Cleaning solution	Any of the following: • Acetic acid • Citric acid • 25–50 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	20% (v/v) ethanol
Operating and storage temperatures	• **Operating**: 2–25°C • **Short-term storage**: Room temperature • **Long-term storage**: 2–8°C

Flow characteristics

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

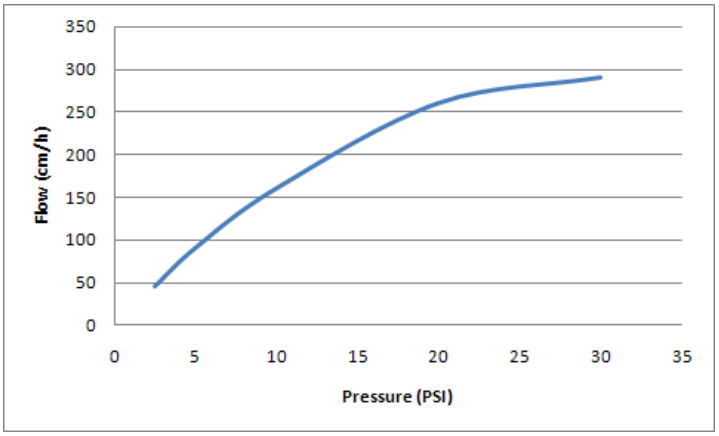

Figure 1 Pressure-flow properties of an agarose-based CaptureSelect™ matrix tested on a 10-cm diameter column packed to 16-cm bed height. The resin can be operated at flow rates up to 300 cm/h, with a pressure drop that allows use in conventional low-pressure chromatography columns and systems.

However, for optimal binding capacity, 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.

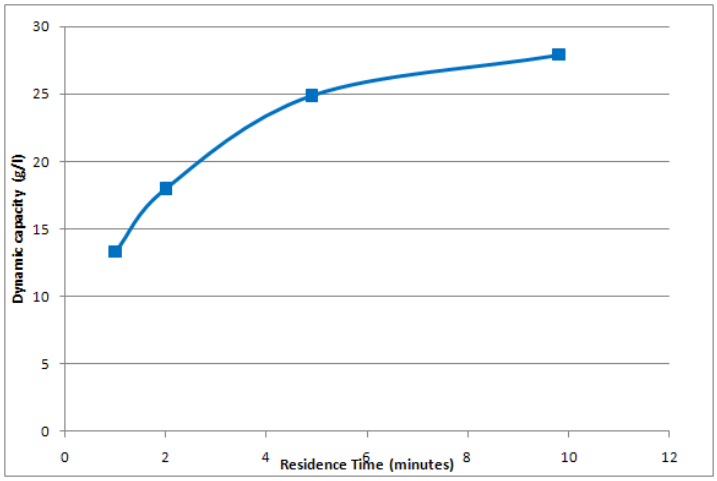

Figure 2 The dynamic binding capacity of the CaptureSelect™ FcXL Affinity Matrix at 10% breakthrough as a function of residence time. The dynamic binding capacity is determined with purified human IgG as a load on a 5-mm x 50-mm column. We recommend residence times of at least 5 minutes.

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 guidelines below 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.

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). The CaptureSelect™ FcXL 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 3).

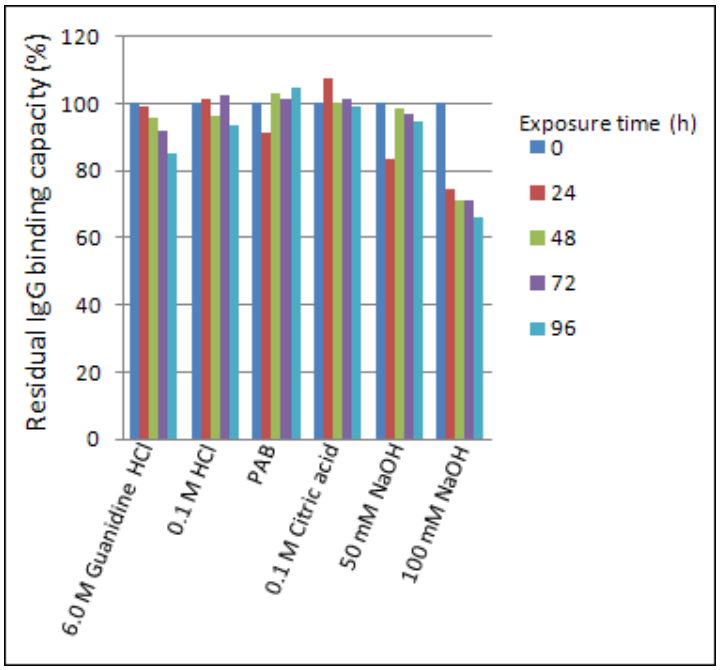

Figure 3 The CaptureSelect™ FcXL Affinity Matrix is compatible with acidic and mildly caustic cleaning agents for up to 96 hours at ambient temperature. In addition, chaotropic agents like guanidine-HCl are compatible with the resin.

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, HER4D5 IgG2 monoclonal was purified from HEK cell feedstock. After the resin was loaded, the column was equilibrated, then eluted. Conditions were as follows:

- **Column** – 0.4-mL CaptureSelect™ FcXL Affinity Matrix packed to a 2-cm bed height
- **Equilibration buffer** – PBS, pH 7.4
- **Load** – 50 mL of clarified cell culture harvest from HEK cells expressing HER4D5 (IgG2) at a titer of 0.08 mg/mL
- **Elution buffer** – 20 mM sodium acetate, 1.0 M $MgCl_2$, 40% (v/v) propylene glycol, pH 5.0
- **Flow** – 200 cm/h

The monoclonal elutes very well under these mild pH conditions. The collected fractions were analyzed on non-reduced SYPRO® Ruby-stained SDS-PAGE, showing highly pure monoclonal antibody in the elution fraction (Figure 4).

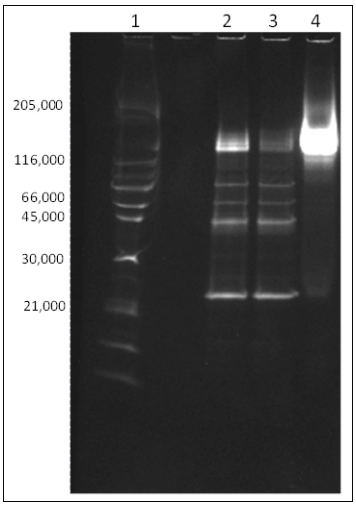

Figure 4 SYPRO® Ruby-stained SDS-PAGE analysis of the fractions from the purification. Over-expressed light chains are present in the flow through and intact monoclonal IgG are eluted from the column under mild pH elution conditions.

Lane 1: molecular weight marker; Lane 2: starting material; Lane 3: flow through; Lane 4: elution

Elution optimization

When co-solvents are used in the elution buffer, you can elute the CaptureSelect™ FcXL Affinity Matrix at milder pH conditions than the standard pH 3.5 acidic elution conditions. In this example, a Design of Experiments (DoE) was performed that combined propylene glycol (5–40%) with $MgCl_2$ (0.1–1.0 M) in sodium acetate buffer at low (pH 3.0), intermediate (pH 5.0), and high (pH 7.0) pH; polyclonal human IgG was the load and the elution efficiency of the buffers was the read out. This experiment resulted in a model for the elution efficiency of the CaptureSelect™ FcXL Affinity Matrix (Figure 5).

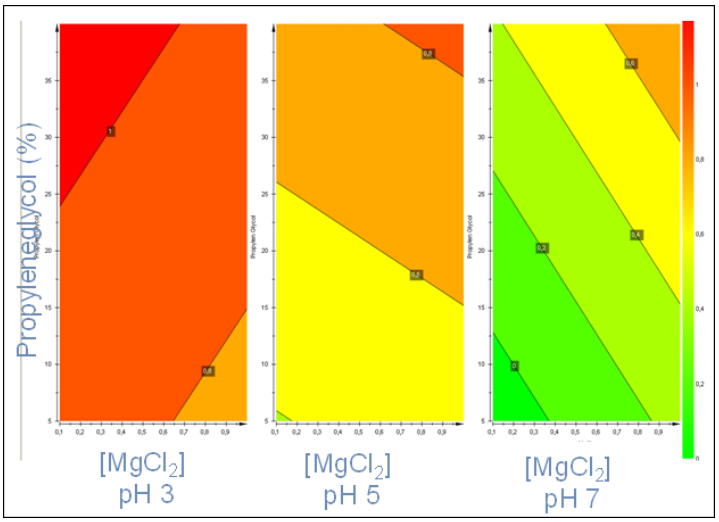

Figure 5 A model for the elution efficiency of the CaptureSelect™ FcXL Affinity Matrix, using co-solvents in the elution buffer. At low pH, we recommend low conductivity buffers; you can add propylene glycol to optimize the elution. At mild and neutral pH, we recommend adding $MgCl_2$ (we tested up to 1.0 M) combined with propylene glycol (we tested up to 40% [v/v]) for efficient elution.

Red: very efficient elution; Green: low elution efficiency

Ordering information

Product	Size	Cat. no.
CaptureSelect™ FcXL Affinity Matrix	250 mL	1943280250
	500 mL	1943280500
	1 L	19432801L
	5 L	19432805L

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 human IgG or Fc fusion proteins. The pre-packed columns include functionalized POROS® 20-µm resin.

A biotinylated anti-IgG Fc (human) conjugate is also available. Applications for the CaptureSelect™ Biotin Anti-IgG-Fc (Hu) 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 (ForteBio® Octet® systems)

The pre-packed columns and the biotinylated anti-IgG Fc (human) conjugate contain a CaptureSelect™ affinity ligand that recognizes exactly the same epitope as the ligand used for the CaptureSelect™ FcXL Affinity Matrix, but the ligand does not have the mild elution characteristics of the CaptureSelect™ FcXL ligand.

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

Product	Size	Cat. no.
POROS® CaptureSelect™ IgG Fc Affinity Column	2.1 × 30 mm	4469148
	4.6 × 50 mm	4469153
	4.6 × 100 mm	4469166
	10 × 100 mm	4469171
CaptureSelect™ Biotin Anti-IgG-Fc (Hu) Conjugate	100 µg	7103262100
	500 µg	7103262500
CaptureSelect™ FcXL Ligand Leakage ELISA Kit	1 assay	810328001
	10 assays	810328010

For more information

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

Customer and technical support

Visit **www.lifetechnologies.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.lifetechnologies.com/termsandconditions**. If you have any questions, please contact Life Technologies at **www.lifetechnologies.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.

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19 February 2015

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