# CaptureSelect™ FcXP Affinity Matrix

Catalog Numbers 1943712250, 194371201L, 194371205L

Pub. No. MAN0019514 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 CaptureSelect™ FcXP Affinity Matrix is specifically developed for the purification of recombinant human IgG, Fc fusion proteins, and plasma-derived IgG through binding the CH3 domain of human IgG. The FcXP ligand (the next generation of CaptureSelect™ FcXL ligand), recognizes all human IgG subclasses (IgG1, 2, 3, and 4) and is designed to obtain increased binding capacity and increased caustic stability. The affinity matrix is human-specific and does not bind IgG from other species (including bovine, horse and rodent).

## Contents and storage

| Product | Cat. No. | Amount | Storage |
|---|---|---|---|
| CaptureSelect™ FcXP Affinity Matrix | 1943712250 | 250 mL | 2–8°C<br>Protect from light. |
| | 194371201L | 1 L | |
| | 194371205L | 5 L | |

## Product advantages

The CaptureSelect™ FcXP 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
- Improved binding capacity and caustic stability

## Specifications

| | |
|---|---|
| Ligand | CaptureSelect™ FcXP Affinity Ligand |
| Binding specificity | Human IgG (all four subclasses) and Fc fusion proteins |
| Matrix and particle size | Epoxide-activated agarose, 65 µm |
| Dynamic binding capacity | >40 g of IgG/L of matrix (10% breakthrough at 5 minutes residence time) |
| Shipping solution | 20% (v/v) ethanol |

Caution: For manufacturing, processing or repacking.

## Conditions for use

| Parameter | Conditions for use |
|---|---|
| Equilibration buffer | 20 mM Tris or PBS, pH 7.0–7.5 |
| Elution buffer | 20 mM acetic acid, pH 4.0–4.5 |
| Strip buffer | Any of the following:<br>• 0.1 M glycine, pH 2.0<br>• 0.5 M acetic acid<br>• 0.5 M citric acid |
| Flow rate | 50–200 cm/h |
| Pressure limit | ≤ 2 bar |
| Cleaning solution | Any of the following:<br>• Acetic acid<br>• Citric acid<br>• 50–100 mM NaOH (Higher concentrations affect the functionality of the affinity ligand on the matrix.)<br>• 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.<br>• 2.0 M guanidine HCl |
| Storage solution | 20% (v/v) ethanol |
| Operating and storage temperatures | • **Operating**: 2–25°C<br>• **Short-term storage**: Room temperature<br>• **Long-term storage**: 2–8°C |

## Flow characteristics

Agarose-based CaptureSelect™ affinity matrices 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 (Figure 1). However, for optimal binding capacity, flow rates of 50–200 cm/h are recommended.

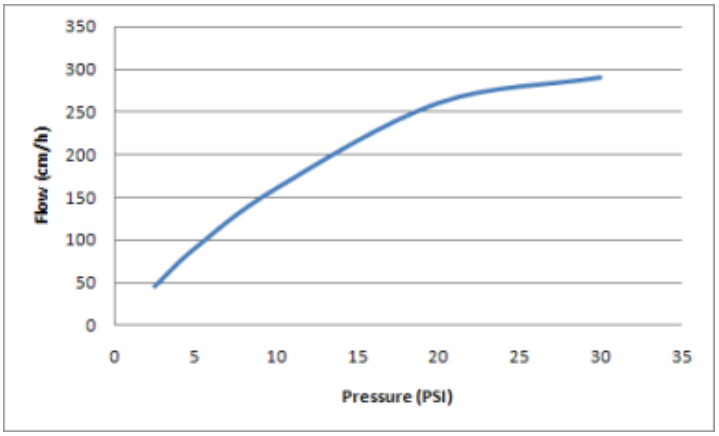


**Figure 1 Pressure-flow properties of an agarose-based CaptureSelect™ matrix tested on a 10-cm diameter column packed to 16-cm bed height.**

Lower flow rates result in longer contact time of the load with the affinity matrix and drives the binding capacity (Figure 2). We recommend residence times of at least 5 minutes.

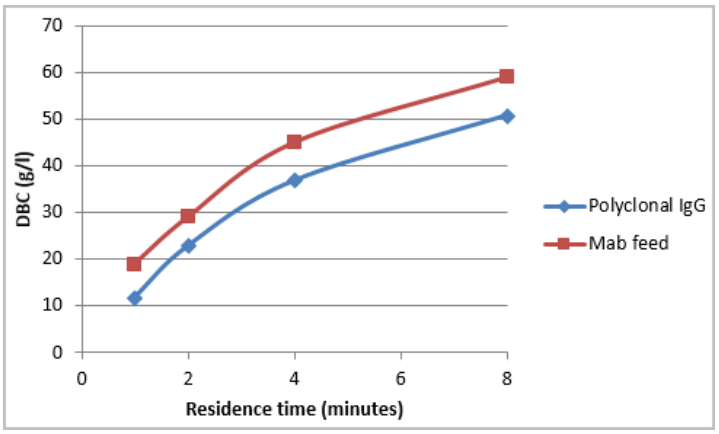


**Figure 2 The dynamic binding capacity of the CaptureSelect™ FcXP Affinity Matrix at 10% breakthrough for human polyclonal IgG and Mab feed as a function of residence time. The dynamic binding capacity is determined on a 0.5 cm×20 cm column.**

It is recommended 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, it is recommended 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™ FcXP Affinity Matrix was exposed to acidic (Figure 3), caustic (Figure 4), and chaotropic (Figure 5) cleaning agents in 5×20 mm columns. The residual binding capacity was measured after 1, 10 and 20 hours incubation time.

Recommended cleaning agents are 0.5M citric acid, acetic acid, or PAB, 50–100 mM NaOH, or 2M guanidine HCl.

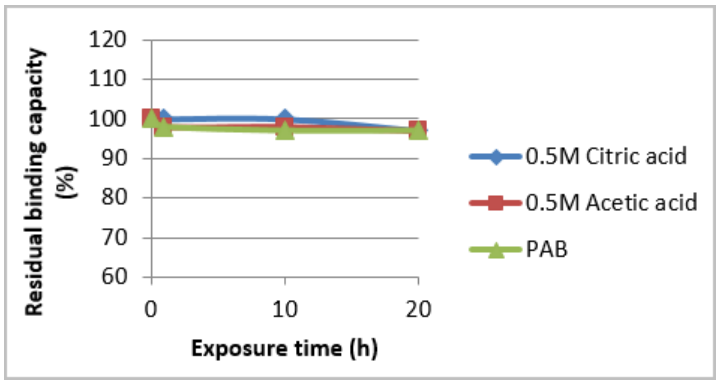


**Figure 3 Residual binding capacity with acidic cleaning agents: citric acid, acetic acid, and PAB solution**

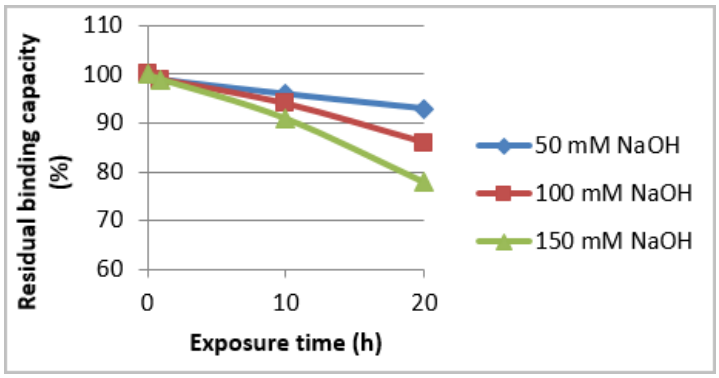


**Figure 4 Residual binding capacity with caustic cleaning agent: NaOH**

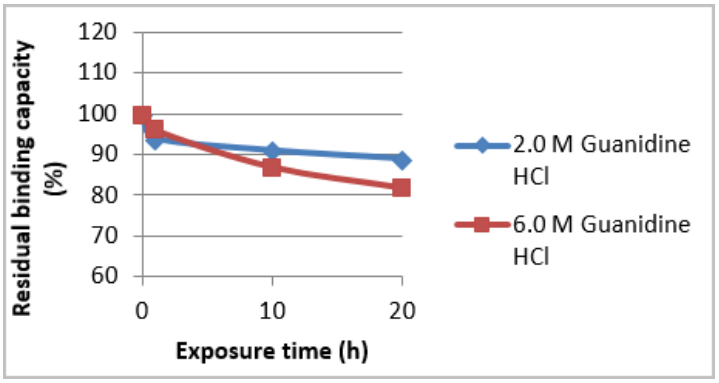


**Figure 5 Residual binding capacity with chaotropic cleaning agent: guandine HCl**

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 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 2.0 M guanidine-HCl can help remove discoloration.

## Example application—Polyclonal human IgG purification

In this example, polyclonal human IgG was purified from undiluted human plasma. The plasma was filtered using a 0.45 µm bottle-top filter and was loaded at room temperature. After the resin was loaded, the column was equilibrated with binding buffer, the bound protein was eluted using a mild elution buffer, then the bound protein was stripped at low pH.

Conditions were as follows:

- **Column**—3.7-mL CaptureSelect™ FcXP Affinity Matrix packed to a 19-cm bed height
- **Equilibration buffer**—10 mM citric acid, 150 mM NaCl, pH 7.4
- **Load**—21 mL of undiluted human plasma with 6.7 g/L IgG titer
- **Elution buffer**—20 mM acetic acid, pH 4.0
- **Strip buffer**—100 mM glycine, pH 2.0
- **Flow**—230 cm/h (5 minute residence time)

The IgG elutes at 99% efficiency at this mild elution condition with a total yield of 90%. Fractions from the purification were taken and analyzed on non-reduced Bio-Rad™ Mini-PROTEAN™ TGX, Stain-Free™ gel, showing high purity of the IgG in the elution (Figure 6).

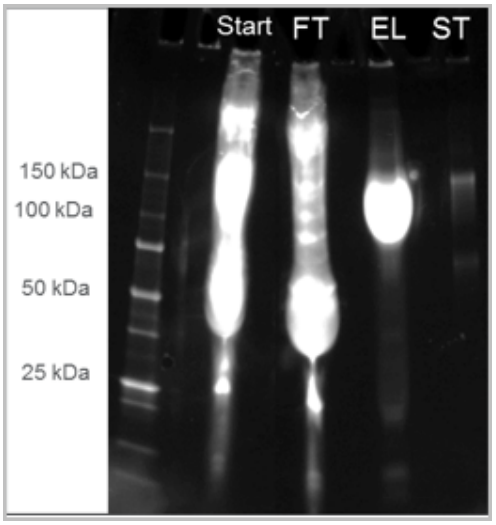


**Figure 6 Bio-Rad™ Mini-PROTEAN™ TGX, Stain-Free™ gel analysis of the fractions from the purification. The elution fraction shows a high purity at mild elution condition with limited loss at the strip condition.**
Start: undiluted human plasma; FT: flow through; EL: elution; ST: strip

## Example application—Rituximab purification

In this example, feedstock of rituximab (a monoclonal human/mouse chimeric IgG1 produced in Expi293 cells (research-grade biosimilar from Thermo Fisher Scientific) was loaded onto the resin. Different amounts of the feed were loaded to determine the dynamic binding capacity of the resin (Figure 7).

After the resin was loaded, the column was equilibrated with binding buffer, the bound protein was eluted using a mild elution buffer, then the bound protein was stripped at low pH. The IgG elutes at 99% efficiency at this mild elution condition.

Conditions were as follows:

- **Column**—4-mL CaptureSelect™ FcXP Affinity Matrix packed to a 20-cm bed height
- **Equilibration buffer**—25 mM Tris, 0.15M NaCl, pH 7.4
- **Load**—Different amounts of clarified cell culture harvest from Expi293 cells expressing rituximab at a titer of 1.7 g/L
- **Elution buffer**—20 mM acetic acid, pH 4.0
- **Strip buffer**—100 mM glycine, pH 2.0

- **Flow**—200 cm/h (6 minute residence time)

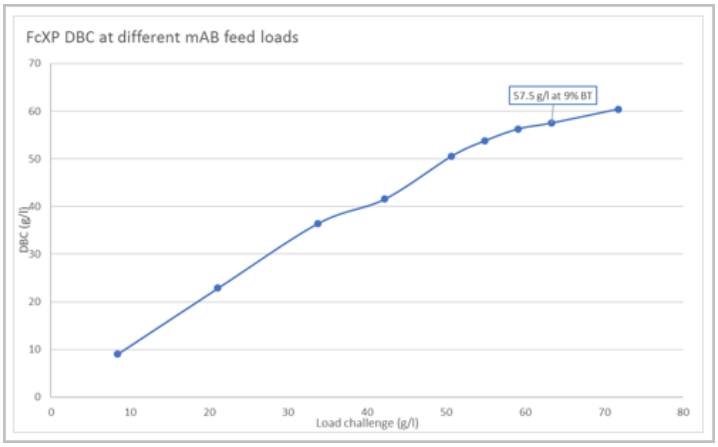


**Figure 7 Dynamic binding capacity of FcXP at different load challenges. A linear increase with high yield and a high binding capacity of 58 g IgG/L resin at 10% breakthrough was observed.**

Fractions from the purification were taken and analyzed on non-reduced Bio-Rad™ Mini-PROTEAN™ TGX, Stain-Free™ gel, showing high purity of the IgG in the elution.

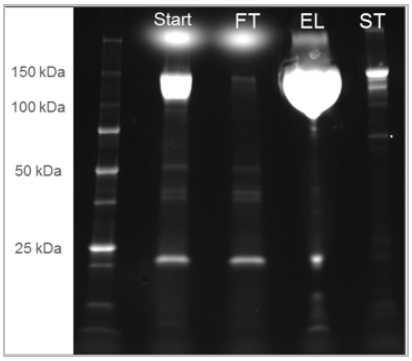


**Figure 8 Bio-Rad™ Mini-PROTEAN™ TGX, Stain-Free™ gel analysis of the fractions from the purification. Over-expressed light chains are present in the flow through and intact monoclonal IgG are eluted from the column under mild pH elution conditions.**
Start: mAB feed; FT: flow through; EL: elution; ST: strip

## 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.

## Supporting products

The FcXP matrix is also available in prepacked column formats:

- **RoboColumn™ format**—For high throughput resin screening and process development. These columns are small chromatography columns that are provided in 8-column strips. The columns are useful for fully automated and parallel chromatographic separations using a robotic liquid handling platform.
- **Mini-Chrom format**—For bench-scale resin screening, process development, and sample preparation.
- **EvolveD™ format**—For for use in biopharmaceutical applications.

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)

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

| Product | Size | Cat. No. |
|---|---|---|
| CaptureSelect™ FcXP RoboColumn™ | 200 µL | 5943712200 |
| CaptureSelect™ FcXP MiniChrom Column | 1 mL | 5943712001 |
| | 5 mL | 5943712005 |
| CaptureSelect™ FcXP EvolveD™ Column | 385 mL, 7×10 cm | 6943712071 |
| | 770 mL, 7×20 cm | 6943712072 |
| | 785 mL, 10×10 cm | 6943712101 |
| | 1,540 mL, 10×20 cm | 6943712102 |
| | 3,142 mL, 20×10 cm | 6943712201 |
| | 6,284 mL, 20×20 cm | 6943712202 |
| CaptureSelect™ Biotin Anti-IgG-Fc (Hu) Conjugate | 100 µg | 7103262100 |
| | 500 µg | 7103262500 |
| CaptureSelect™ FcXP Ligand Leakage ELISA | 1 assay | 810371201 |
| | 10 assays | 810371210 |

## For more information

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

## Customer and technical support

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- 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. MAN0019514

| Revision | Date | Description |
|---|---|---|
| A.0 | 28 August 2020 | New document for CaptureSelect™ FcXP Affinity Matrix. |