CaptureSelect™ IgG-CH1 Affinity Matrix

Catalog Number 1943200250, 1943200500, 19432001L, 19432005L

Pub. No. MAN0009646 **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 **www.lifetechnologies.com/support**.

Product description

The CaptureSelect™ IgG-CH1 Affinity Matrix purifies recombinant human Fab fragments and IgG from complex source materials (such as cell culture medium) in a single step. The affinity matrix recognizes all four subclasses of IgG (IgG1, IgG2, IgG3, and IgG4), independent of the light chain type (kappa/lambda).

The matrix combines selectivity for the CH1 domain on the heavy chain of human IgG 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™ IgG-CH1 Affinity Matrix offers:

- A platform for purifying human Fab fragments and IgG, without co-purifying over-expressed light chains and light chain dimers in Fab productions
- High recovery and purity in a single step
- Compatibility with FPLC systems

Specifications

Ligand	CaptureSelect™ IgG-CH1 Affinity Matrix
Binding specificity	Human Fab- and Fab_2 fragments and human IgG (all four subclasses)
Matrix and particle size	Aldehyde-activated agarose, 65 µm
Dynamic binding capacity	22 g of IgG/L of matrix (10% breakthrough at 10 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	20 mM acetic acid or citric acid, 150 mM NaCl, pH 3.5
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 • 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	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).

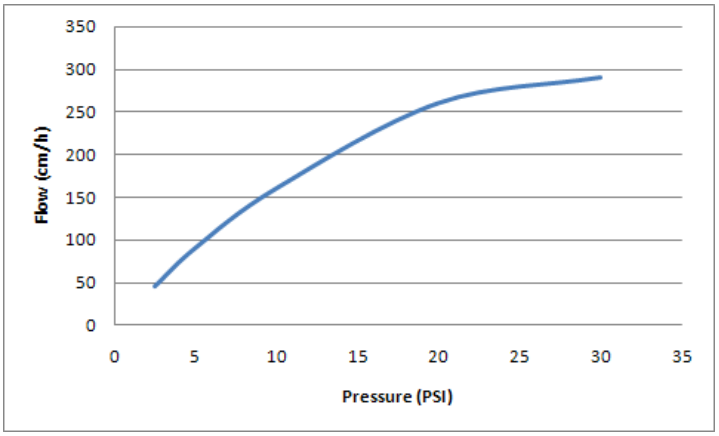

Figure 1 Pressure-flow properties of an agarose-based CaptureSelect™ matrix tested on a 10-cm diameter column packed to 16-cm bed height. The resin can be operated at flow rates up to 300 cm/h, with a pressure drop that allows use in conventional low-pressure chromatography columns and systems.

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.

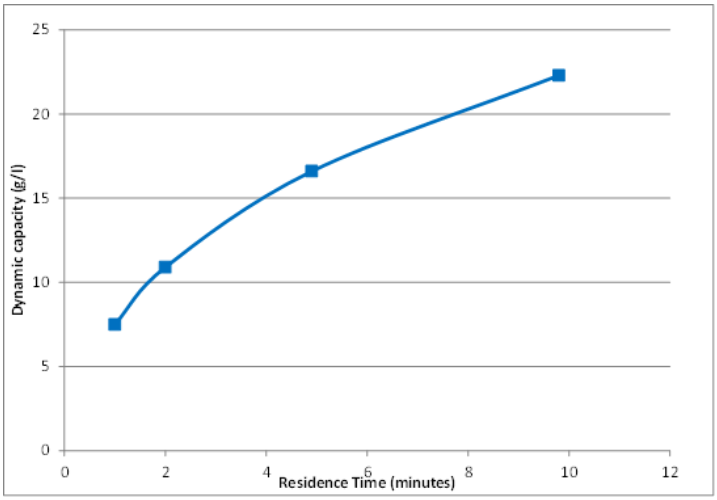

Figure 2 The dynamic binding capacity of the CaptureSelect™ IgG-CH1 Affinity Matrix at 10% breakthrough as a function of residence time. The dynamic binding capacity is determined with purified human IgG as a load on a 5-mm x 50-mm column. For high dynamic binding capacity, we recommend residence times of 10 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 - FPLC

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™ IgG-CH1 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).

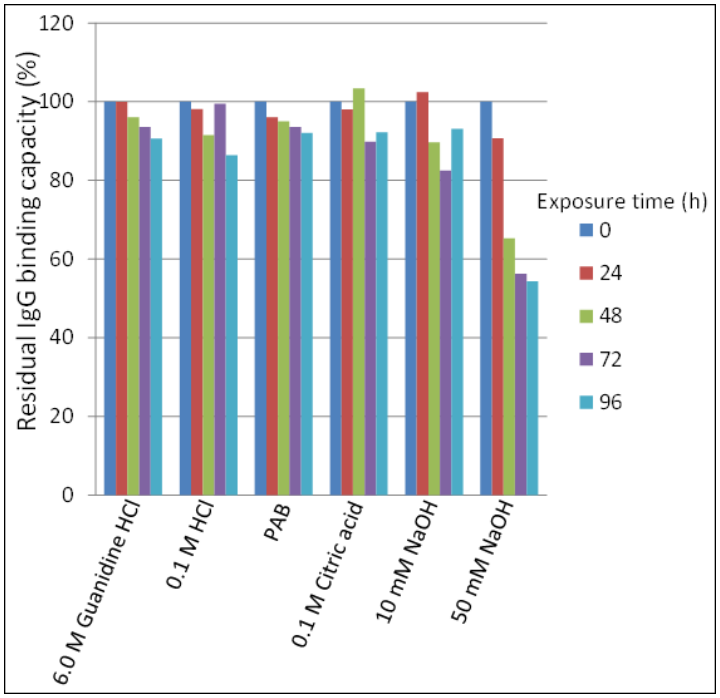

Figure 3 The CaptureSelect™ IgG-CH1 Affinity Matrix is compatible with acidic and mildly caustic cleaning agents for up to 96 hours at ambient temperature. In addition, chaotropic agents like guanidine-HCl are compatible with the resin.

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 cells. After the resin was loaded, the column was equilibrated, then eluted. Conditions were as follows:

- **Column** – 0.4-mL CaptureSelect™ IgG-CH1 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 citric acid, 150 mM NaCl, pH 3.5
- **Flow** – 200 cm/h

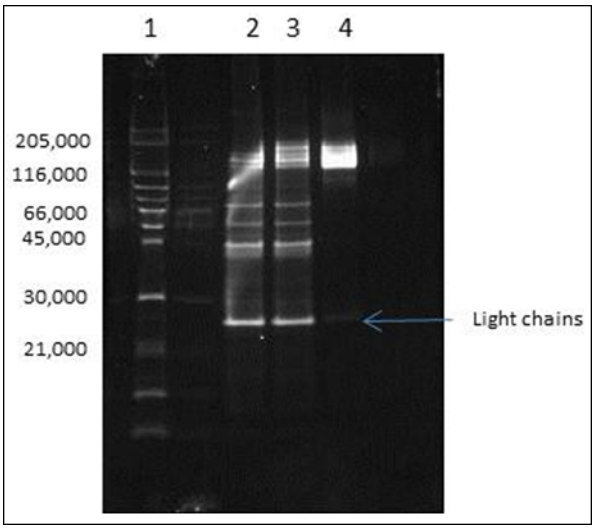

Figure 4 SYPRO® Ruby-stained SDS-PAGE analysis of the fractions from the purification. Over-expressed light chains are present in the flow through and intact monoclonal IgG are eluted from the column.

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

Ordering information

Product	Size	Cat. no.
CaptureSelect™ IgG-CH1 Affinity Matrix	250 mL	1943200250
	500 mL	1943200500
	1 L	19432001L
	5 L	19432005L

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

A biotinylated anti-IgG-CH1 (human) conjugate is available. Applications for the CaptureSelect™ Biotin Anti-IgG-CH1 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™ IgG-CH1 Affinity Matrix.

Product	Size	Cat. no.
CaptureSelect™ Biotin Anti-IgG-CH1 Conjugate	100 µg	7103202100
	500 µg	7103202500
CaptureSelect™ IgG-CH1 Ligand Leakage ELISA Kit	1 assay	810320001
	10 assays	810320010

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

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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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