Introduction

The CaptureSelect human Fc affinity matrix contains a 13 kDa Llama antibody fragment recognizing human IgG. The used ligand is directed towards a unique domain on human Fc that enables purification of all subclasses (including IgG3) of human IgG's, providing improved performance over Protein A. The ligand is coupled to NHS-activated Sepharose 4 Fast Flow. This matrix can be used for the purification and isolation of human IgG from complex sources like plasma, serum, and cell culture supernatants. The matrix can also be used for the purification of recombinant Fc fusion proteins.

Characteristics of CaptureSelect human Fc

Dynamic binding capacity:	>10 mg IgG per ml matrix (linear flow rate 150 cm/h)
Beads:	NHS-activated Sepharose 4 Fast Flow (GE Healthcare)*
Average particle size:	90 µm
Ligand coupling method:	N-Hydroxysuccinimide (NHS) activation
Elution conditions:	Acidic elution with 0.1 M Glycine pH 3.0 or 2.0
Short term storage:	20% ethanol at room temperature
Long term storage:	20% ethanol at 4 °C, stable for 1 year

* For more information about NHS-activated Sepharose 4 fast flow and column packaging see information on the website of GE Healthcare (http://www.gehealthcare.com) product code: 17-0906-02

Application

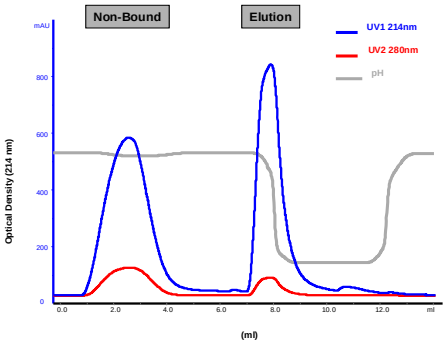
A typical chromatography protocol for the CaptureSelect human Fc matrix is:

1) Carefully pack the CaptureSelect affinity matrix in a column and equilibrate the matrix by adding a suitable binding buffer. A good general binding buffer is PBS, pH 7.2-7.4 (physiological pH & ionic strength). Ultimately, the most suitable binding conditions need to be determined empirically.
2) Sample can be applied on the column. The optimal binding condition for samples is around physiological pH. Samples with acidic pH should be avoided, since binding of the target molecule will not occur at these conditions. The amount of sample that can be loaded is depending on the concentration of the target molecule in your sample and the dynamic binding capacity of the matrix. The dynamic binding capacity for human IgG is 10 mg per ml matrix, using a linear flow of 150 cm/h.
3) After sample application, the column should be washed with binding buffer until baseline has been re-established on the monitor. A typical wash is 5-10 column volumes.
4) Elution of the target molecule from the affinity media is achieved by an acidic elution buffer, 5 column volumes (CV) of 0.1 M Glycine pH 3.0 is recommended.

5) The affinity matrix should be re-equilibrated in binding buffer and is ready to use for a second affinity purification run. If the column will not be used immediately, the matrix should be stored in 20% ethanol at 4 °C (39 °F).

A typical example of a chromatography run with CaptureSelect affinity matrices

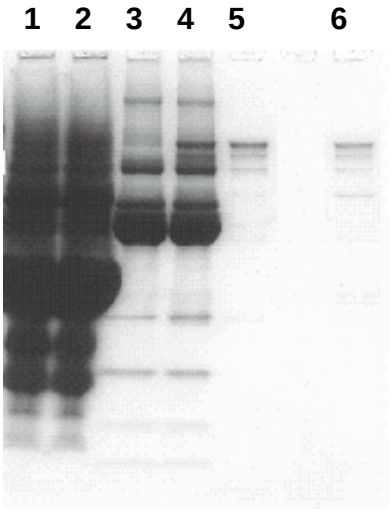
Equilibration and washing buffer: PBS pH 7.4
Elution buffer: 0.1 M Glycine pH 3.0
Flow: 150 cm/h



1 Cycle is: -10 column volumes (CV) equilibration
- sample loading
- 10 CV wash out unbound sample
- 5 CV elution
- 10 CV re-equilibration

SDS-PAGE of the purification of human IgG from CHO cell culture medium and bovine milk

Samples from the purification of human IgG from bovine milk and CHO cell culture medium were analyzed by SDS-PAGE.



1 bovine milk
2 bovine milk spiked with human IgG
3 CHO cell culture medium
4 CHO cell culture medium spiked with human IgG
5 elution fraction from bovine milk
6 elution fraction from CHO cell culture medium

Human IgG was purified in one single step from complex mixtures of proteins like bovine milk and cell culture medium.

The Toolbox for Antibody Purification

The CaptureSelect antibody toolbox product line consists of seven different affinity matrices that can bind human IgG, human IgG4, human IgA, human kappa Ig light chains, and human lambda Ig light chains. The IgM affinity matrix is specific for human and mouse IgM, and a multi species IgG affinity matrix that can bind different species IgG completes our antibody purification toolbox.

Contact

For further information or questions about the antibody toolbox matrices please send an E-mail to ligands@captureselect.com