Introduction

The CaptureSelect Fab lambda affinity matrix contains a 13 kDa Llama antibody fragment recognizing human lambda light chain with high affinity. The used ligand is directed towards a unique domain on the lambda light chain of human Ig's that enable purification of human IgG, IgA, IgM, and IgE. The specificity of CaptureSelect Fab lambda affinity matrix is unique, since other proteins that can bind human light chains, Protein L, only bind kappa light chains. In combination with CaptureSelect Fab kappa affinity matrix, full human Ig purification is accomplished. Fab lambda affinity ligand is coupled to NHS-activated Sepharose 4 Fast Flow. This matrix can be used for the purification and isolation of human Ig's from complex sources like plasma, serum, and cell culture supernatants.

Characteristics of CaptureSelect Fab lambda

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 pH 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

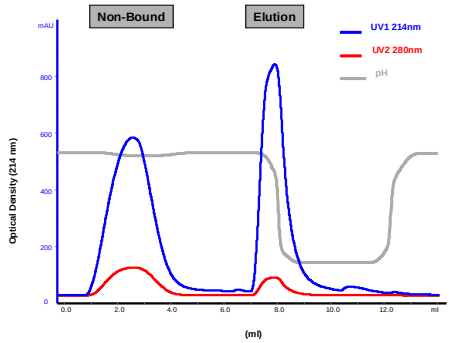
A typical chromatography protocol for the CaptureSelect Fab lambda matrix is as follows;

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



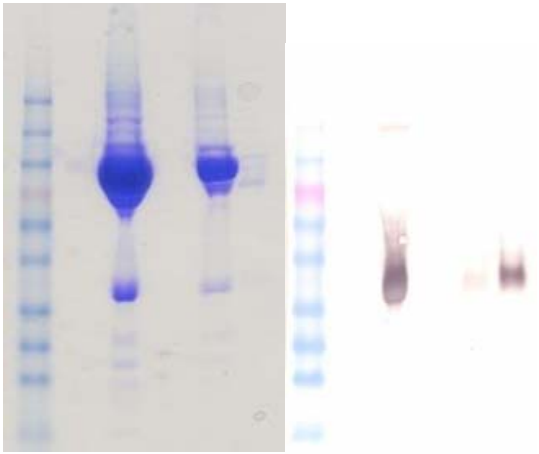
1 Cycle is: -10 column volumes (CV) equilibration
- sample loading
- 10 CV wash out unbound sample
- 5 CV elution
- 10 CV re-equilibration

Purification of lambda Ig's from human plasma

Human plasma was loaded onto CaptureSelect Fab lambda matrix using the described protocol. Human plasma, the non-bound fraction, and elution pool were tested for the presence of lambda light chains using SDS-PAGE and Western blot analysis with specific antibodies against human Fab lambda.

SDS-PAGE and Western blot of Fab lambda purification samples with CaptureSelect Fab Lambda

1 2 3 4 1 2 3 4



Lane 1: Pre-stained marker
Lane 2: human plasma
Lane 3: Non-bound fraction
Lane 4: Elution

Blot: anti-human Fab lambda probe

In one step human lambda Ig's were isolated from plasma.

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