Introduction

The CaptureSelect IgM affinity matrix contains a 14 kDa Llama antibody fragment recognizing IgM. The used ligand is directed towards a unique domain on the Fc part of IgM that enables purification of human and mouse IgM's without cross reactivity with human and mouse IgG and IgA. This makes the CaptureSelect IgM affinity matrix a superior resin in comparison with other IgM matrices in terms of specificity and purity. The ligand is coupled to NHS-activated Sepharose 4 Fast Flow. This matrix can be used for the purification and isolation of IgM, without cross-binding to other immunoglobulins, from complex sources like plasma, serum, and cell culture supernatants.

Characteristics of CaptureSelect IgM affinity matrix

Dynamic binding capacity:	>2.5 mg IgM 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
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

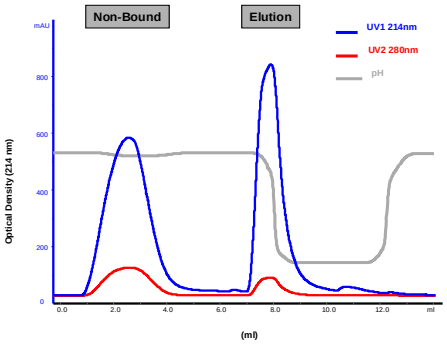
A typical chromatography protocol for the CaptureSelect IgM matrix is:

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 IgM is at least 2.5 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

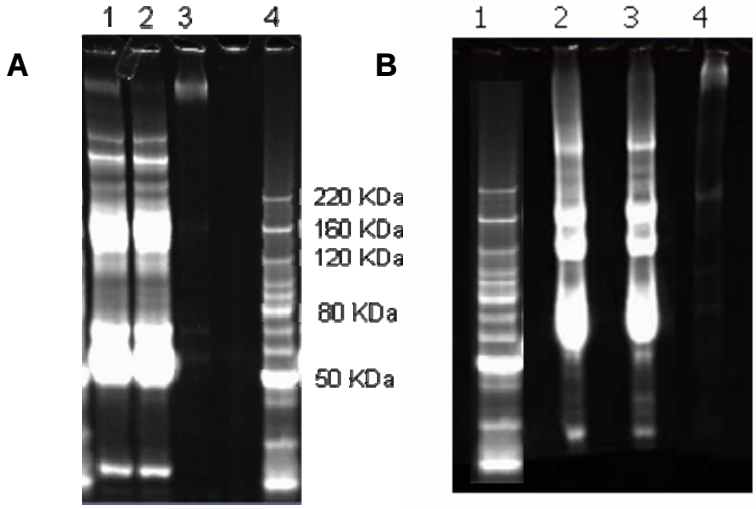
Equilibration and washing buffer: PBS pH 7.4
Elution buffer: 0.1 M Glycine pH 3.0
Flow: 150 cm/h



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 IgM from human and mouse serum

200 µl human (A) and mouse serum (B) was loaded on 400 µl CaptureSelect IgM affinity matrix with a flow of 150 cm/h. The columns were washed with 10 CV PBS pH 7.4 and eluted with 5 CV 0.1 M glycine pH 3.0. The elution fractions were neutralized with 0.1 volume 1 M Tris pH 8.0. The starting material, flow through, and elution fractions were analyzed on a Sypro ruby stained non-reduced 4% acrylamide tris glycine gel.



A 1: human serum
2: flow through IgM affinity matrix
3: elution IgM affinity matrix
4: molecular weight marker

B 1: molecular weight marker
2: mouse serum
3: flow through IgM affinity matrix
4: elution IgM affinity matrix

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