



## Introduction

The CaptureSelect Human IgG4 affinity matrix contains a 12 kDa Llama antibody fragment recognizing human IgG4. The used ligand is directed towards a unique domain on the Fc part of the human IgG subclass 4 that enables purification of human IgG4's without cross reactivity with human IgG from subclasses 1, 2, and 3. This makes the CaptureSelect Human IgG4 affinity matrix a unique resin in terms of specificity. The ligand is coupled to NHS-activated Sepharose 4 Fast Flow. This matrix can be used for the purification and isolation of IgG4, without cross-binding to other immunoglobulins, like other Human IgG subclasses, IgA and IgM, from complex sources like plasma, serum, and cell culture supernatants.

## Characteristics of CaptureSelect Human IgG4 affinity matrix

Dynamic binding capacity:	>6 mg IgG4 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 Human IgG4 matrix is:

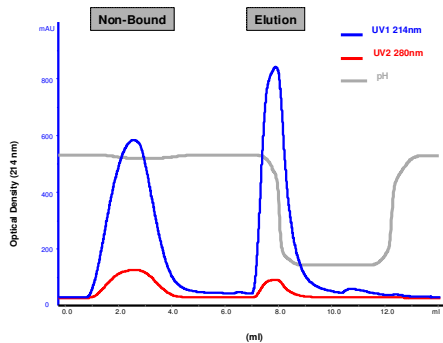
- 1) Carefully pack the CaptureSelect affinity matrix in a column and equilibrate the matrix by adding a suitable binding buffer. A good general binding buffer is PBS, pH 7.2-7.4 (physiological pH & ionic strength). Ultimately, the most suitable binding conditions need to be determined empirically.
- 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 IgG4 is at least 6 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.

**Warranty:** Antibody toolbox affinity matrices of BAC BV are supplied for research use only and are intended to be used by a technically qualified individual. BAC BV makes no claim of suitability for use of antibody toolbox affinity matrices in applications regulated by FDA. If you are not satisfied with the performance of a BAC BV product, please contact BAC BV.

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 Human IgG4 from serum

Human serum was loaded on CaptureSelect human IgG4 affinity matrix. After washing, the matrix was eluted with 0.1 M Glycine pH 3.0. The starting material and elution fraction were analyzed on subclass distribution of human IgG. The elution fraction contains more than 97% IgG4, with small amounts of IgG1 and IgG3.

	IgG subclass (%) starting material	IgG subclass (%) in elution fraction
IgG 1	57.1	1.7
IgG 2	34.2	0
IgG 3	4.0	0.6
IgG 4	4.7	97.7

→ **Large reduction in IgG1, 2, 3:  
 Good enrichment for IgG4**



## **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](mailto:ligands@captureselect.com)