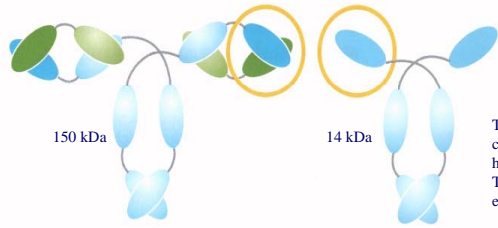


Caustic Stable Human IgG Capture Ligand



The difference between classical antibodies (left) and heavy chain antibodies (right). The binding domain is encircled in yellow

Introduction

Affinity chromatography is a well-established technology for purifying biological molecules from complex source materials. The most commonly used application of large-scale affinity chromatography is monoclonal antibody purification by bacterial coat protein (Protein A) resins. The widespread use of affinity purification in large scale downstream processing has been hampered by the absence of safe affinity ligands that fulfill the needs of large scale affinity chromatography including chemical stability, "tunable" affinity, high selectivity, short development times, and cost in use.

We have developed a technology that fulfils these basic demands by making use of Camelidae heavy chain antibody fragments. BAC's CaptureSelect® ligand technology is based on the rapid identification of highly stable and specific affinity ligands using immune antibody libraries efficiently expressing these heavy chain antibody fragments in the yeast *S. cerevisiae*. These ligands can be used for generic purification solutions such as Human IgG, HSA and Human Transferrin. More importantly, CaptureSelect® ligands can be custom made to solve virtually any biotherapeutic purification challenge.

CaptureSelect® caustic stable Human IgG ligand

From an immune library, specific clones producing heavy chain antibody fragments were screened against Human IgG. Positive clones were screened for stability at concentrations of sodium hydroxide using ELISA and surface plasma resonance on a BiaCore 3000. Selected ligands were produced using BAC's *S. cerevisiae* plug-in cloning system. The selected ligands were screened for chromatographic behavior, and the best performing ligands were further characterized for binding affinity to Human IgG subclasses and IgG from other species. Also the stability towards caustic of the immobilized ligand was tested.

SPR Affinity	Kd
Human IgG1	5.8 * 10 ⁻¹⁰
Human IgG2	1.7 * 10 ⁻⁹
Human IgG3	4.8 * 10 ⁻⁸
Human IgG4	7.9 * 10 ⁻¹⁰

Subclass and species specificity of the ligand for Human IgG were determined using SPR on a BiaCore 3000. No cross-reactivity with Mouse IgG or Bovine IgG was found. The ligand binds all Human IgG subclasses.

The ligand immobilized onto NHS sepharose showed excellent performance when incubated with sodium hydroxide. No loss of dynamic binding capacity was found after incubation for 120 cycles of 15 minutes each at 0.1M NaOH. At 0.2M NaOH the matrix could withstand 60 incubation of 15 minutes without loss of dynamic binding capacity. During all incubations with sodium hydroxide, no changes in peak symmetry were found.

Purification of IgG from tissue culture

Over the past decades the purification of Human IgG usually involved an affinity capture step with protein A. Various drawbacks are associated with this step. One of these drawbacks is the change in dynamic binding capacity over the different subclasses of IgG, and pI of the antibodies. The CaptureSelect® affinity ligand shows identical dynamic binding capacities over the different subclasses and pI's, as can be seen from the table below.

Sample	Dynamic capacity (mg IgG/ml matrix, 10% Breakthrough)	Flow (cm/hr)
IgG1	25.2	150
IgG2 (pI 7-8)	26.9	200
IgG2 (pI 7-8)	23.6	300
IgG2 (pI 11+)	24.0	150

Consistent dynamic binding capacities over different IgG subclasses and pI's

NHS sepharose immobilized human IgG caustic stable ligand
source material: IgG in cell culture media
Eq buffer PBS pH 7.4, elution buffer: 0.1M HCl/Glyc pH 3.0

Purification of IVIG from Human Plasma

The caustic stable Human IgG ligand can be used for the purification of IVIG. Two important aspects in the purification of IVIG are the purity of the end product (e.g., no Albumin and IgA present) and identical subclass distribution from the starting material to the final product. In collaboration with Baxter Healthcare (Westlake Village, California) IVIG was purified from fibrinogen free plasma. The tables below show the removal of Albumin and IgA during the affinity purification step and the subclass distribution of starting material and elution pool.

	Albumin mg/ml	IgG mg/ml	IgA mg/ml	Albumin recovery(%)	IgG recovery (%)	IgA recovery (%)
Load	15.35	2.58	1.24	100	100	100
Total FT	14.65	0.19	1.13	95.4	7.4	91.1
Wash 1	2.85	0.18	0.2	9.7	3.6	8.4
Wash 2	<0.01	0.08	<0.01	0	1.6	<0.4
Elution pH 3	<0.01	4.15	<0.01	0	87.6	0.2

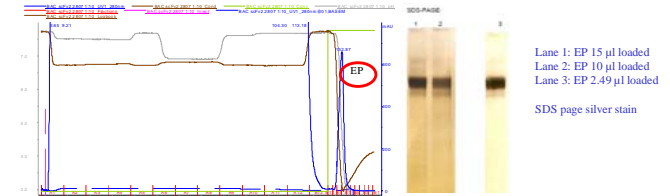
Dynamic capacity: 25 mg/ml
NHS sepharose immobilized human IgG caustic stable ligand
source material: fibrinogen free plasma
process: 25 mg/ml load, 100 cmh, wash 5CV, eluate 1, 5CV, eluate 2, 5CV
Eq buffer PBS pH 7.4, elution buffer 1, 0.1M HCl/Glyc pH 3.0, elution buffer 2, 0.1 M HCl/Glyc pH=2.0

	Cryo Rich Plasma (1)	Eluate Pool (1)	Cryo Rich Plasma (2)	Eluate Pool (2)
IgG 1	41.1%	42.7%	37.4%	42.3%
IgG 2	49.8%	52.2%	56.4%	50.9%
IgG 3	3.1%	2.1%	2.1%	2.1%
IgG 4	5.9%	3.1%	4.2%	4.8%

IVIG purification: Matching subclass distribution in sample and eluate

Purification of IgG2 from Alfalfa

In collaboration with the NRC Biotechnology Research Institute (Montreal, Quebec), the caustic stable human IgG ligand was used for the purification of IgG2 from Alfalfa. The silver stained SDS page gel shows a high purity end product after the capture step.



Column (HR10/2): bed height: 1.95 cm; bed volume: 1.5 ml
NHS sepharose immobilized human IgG caustic stable ligand
Dynamic capacity: 25 mg/ml
Load: 120 ml of pre-treated alfalfa extract containing Human IgG2 in 50 mM Tris-HCl + 150 mM NaCl, pH 8.0 (Buffer A); 0.8 ml/min flow rate
Elution: 100 mM glycine-NaOH, pH 3.0; 2.0 ml/min
Eluate pool (EP): B10-B6 (10.0 ml neutralized with 1.0 ml of 1 M potassium phosphate buffer, pH 7.5)
CIP: 3CV x 100 mM glycine-NaOH, pH 2.0; 0.1 N NaOH for 15 min; Buffer A to pH 8

One step purification of IgG2 from transgenic plants

Purification of IgG from transgenic animals

The production of Human IgG can also be achieved by using transgenic animals. For Purification, a specific Human IgG ligand is then used to separate the host IgG from the Human IgG. The table below compares the percentage of host versus human IgG in the load and elute fraction when the CaptureSelect® caustic stable Human IgG ligand is used.

Sample Load	Human IgG in eluate (mg)	Host IgG in eluate (ppm)
25 mg Human IgG 5 mg Host IgG (20%)	22.75	5426 (0.54%)
50 mg Human IgG 10 mg Host IgG (20%)	43.16	5252 (0.52%)
75 mg Human IgG 15 mg Host IgG (20%)	64.21	5946 (0.59%)
100 mg Human IgG 20 mg Host IgG (20%)	83.19	4764 (0.47%)

NHS sepharose immobilized human IgG caustic stable ligand
Dynamic capacity: 25 mg/ml
Source material: IgG produced by transgenics
Eq buffer PBS pH 7.4, elution buffer: 0.1M HCl/Glyc pH 3.0

Pure IgG from transgenic animals, no host IgG contaminant

CaptureSelect® Human IgG caustic stable ligand: the optimal choice for all IgG purification