IgSelect affinity medium

IgSelect is an affinity medium designed for the purification of human IgG from different sources. The product is a complement to other affinity media with immobilized protein A, such as MabSelect™, since it is specific for human IgG and also binds IgG_3. This chromatography media is developed as a Custom Design Media, and made available for the general market. The benefits of working with IgSelect include:

- Binding all subclasses of human IgG, including IgG_3
- Specificity for human IgG, no cross reaction with IgG from other species
- Rigid base matrix to allow high flow rates
- Availability as prepacked HiTrap™ columns for simple operation with a syringe, pump or chromatographic system.
- Animal free production

Characteristics of the medium

The main characteristics of IgSelect are shown in Table 1. The medium is based on a cross-linked high flow agarose matrix, which allows rapid processing of large sample volumes. The ligand, a 14 kD recombinant protein, is attached to the base matrix with a long, hydrophilic spacer arm to facilitate binding of IgG (Fig. 1). The ligand is coupled with multipoint attachment through stable amide bonds to give high chemical stability and low leakage.

The ligand has been designed for capture of human IgG in a wide range of applications, such as plasma fractionation and purification of human antibodies from different species. The absence of cross reactions with IgG from other species makes the medium excellent for purification of human IgG from transgenic sources. The ligand is based on a single chain antibody fragment that was developed by screening for chemical stability and unique selectivity for Fc fragments of human IgG.

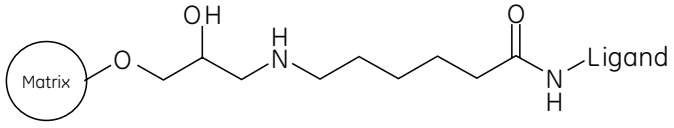


Fig 1. Partial structure of IgSelect

Table 1. Main characteristics of IgSelect

Matrix	Highly cross-linked high flow agarose
Average particle size	75 µm
Ligand	14 kD recombinant protein produced in S. Cerevisiae. Binds to Fc fragments of all human IgG subclasses
Dynamic binding capacity	17 mg/ml at 2.4 min residence time
Flow velocity	At least 600 cm/h in a 1 m column with 20 cm bed height at 20°C using process buffers with the same viscosity as water at < 3 bar (0.3 MPa)
pH stability	
Long term	2–11
Short term	1–13

Principles

Affinity chromatography exploits an immobilized ligand that adsorbs a specific molecule or group of molecules under suitable binding conditions and desorbs them under suitable elution conditions. These conditions depend on the target molecule, feed composition, and chromatography medium, and must be studied together with other chromatographic parameters, such as, sample load, flow velocity, bed height to establish the optimal conditions for the highest product recovery.

IgSelect can be used in the same way as protein A media. Typically, loading, washing and elution of IgG from the column are applicable with conventional buffers such as phosphate-buffered saline solution (PBS), Tris and citrate. Binding of IgG to the column occurs around neutral pH and elution can be done by lowering pH, for example to pH 3-4. Since IgG can be sensitive to very acidic conditions, it is important to minimize the exposure to low pH (< pH 3) during elution. Therefore, we recommend that elution fractions are neutralized immediately.

Depending on the nature of the sample, regeneration is normally performed after each cycle followed by re-equilibration in start buffer. To prevent build up of contaminants over time, more rigorous protocols may have to be applied (see Cleaning-in-place and sanitization).

Breakthrough curve

The binding capacity at 10% breakthrough is typically around 17 mg/ml when residence time is 2.4 min (Fig 2). Longer residence times increase the capacity, for example, at 6 min residence time the capacity exceeds 22 mg/ml.

Column:	Tricorn™ 5/100, 10 cm bed height
Sample:	human IgG
Buffer A:	20 mM sodium phosphate, 150 mM NaCl, pH 7.4
Buffer B:	0.1 M glycine, pH 3.0
Flow rate:	250 cm/h, corresponding to 2.4 min residence time
Dynamic binding capacity:	15.5 mg/ml

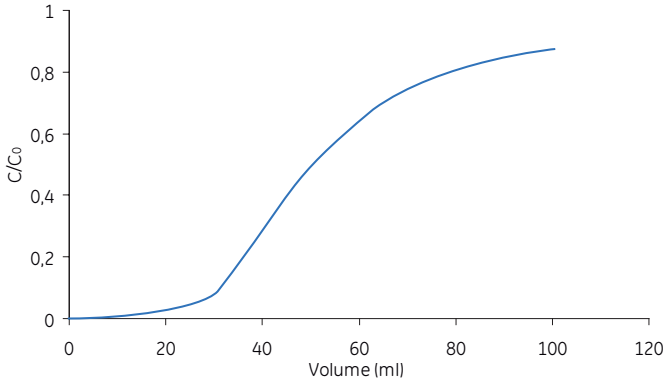


Fig 2. Breakthrough curve of IgSelect.

Purification of IgG from serum

Since IgSelect binds all four subclasses of human IgG it can be an alternative for production of different immunoglobulin preparations from plasma. In this example the one-step purification of IgG from human serum was investigated. Two different volumes of serum containing amounts of IgG corresponding to 70 and 80% of the dynamic binding capacity were loaded in Run 1 and Run 2 respectively (Table 2). The flow rate throughout the purification was 250 cm/h, corresponding to a residence time of 2.4 min (Fig 3). Subclass distributions of eluate fraction and load material were determined by ELISA (Table 3) and purity of eluted IgG was monitored with gel electrophoresis (Fig 4).

Column:	Tricorn 5/100, 10 cm bed height
Flow rate:	250 cm/h, corresponding to 2.4 min residence time
Loading material:	Human serum
Equilibration:	20 mM phosphate, 150 mM NaCl, pH 7.4, 10 column volumes
Wash:	Same as equilibration buffer, 7 or 5 column volumes for Run 1 and Run 2 respectively
Elution:	0.1 M glycine, pH 3.0 in 5 column volumes

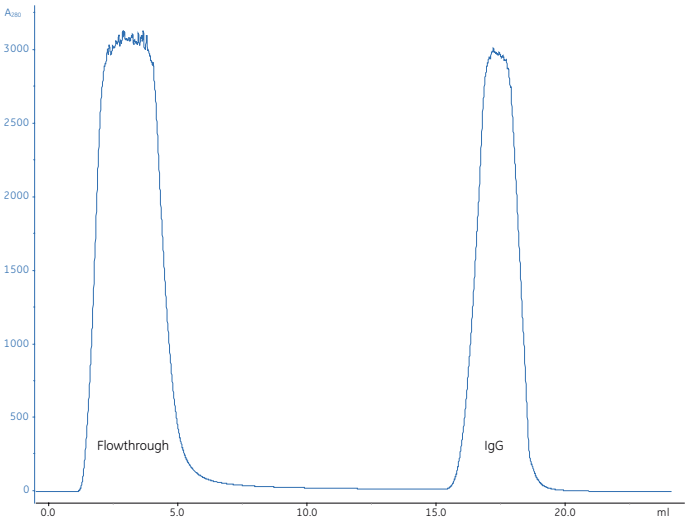


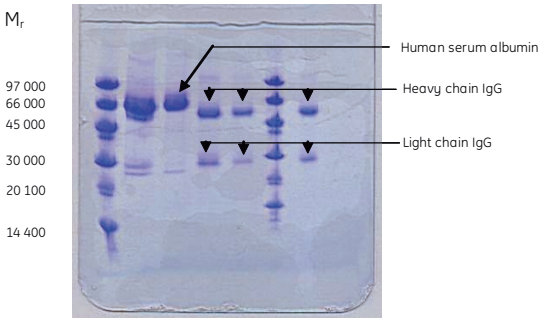
Fig 3. Purification of IgG from human serum.

Table 2. Loaded and eluted amounts of IgG.

	Loaded amount of IgG (mg/ml medium)	Eluted amount of IgG (mg/ml medium)	Yield (%)
Run 1	12.0	10.8	90
Run 2	13.5	12.8	94

Table 3. Sublclass distribution determined by ELISA.

	IgG_1	IgG_2	IgG_3	IgG_4
Run 1, elution fraction	62.3	28.2	5.9	3.7
Run 2, elution fraction	nd	nd	nd	nd
Load material	62.6	28.4	5.9	3.2



Lane
1 LMW
2 Serum
3 Wash
4 Elution diluted 1:5
5 Elution diluted 1:10
6 LMW
7 Commercial IVIG

Fig 4. SDS-PAGE of Run 2. Gradient gel 8–25% under reducing conditions.

The results show that IgG with comparable purity to a commercial IgG preparate can be obtained in one chromatography step. Albumin and other plasma proteins were found in the wash fraction while IgG was eluted with >90% yield in a narrow peak. The subclass distribution in the loading material was retained during the purification.

Stability

The ligand is linked to the agarose base matrix with stable amide bonds. When IgSelect was stored at room temperature for one week at different pH it was found that the ligand leakage is low in the range pH 1 to 12, (Fig. 5). At pH values greater than 12 both carbon and nitrogen is released which indicates hydrolysis of the ligand.

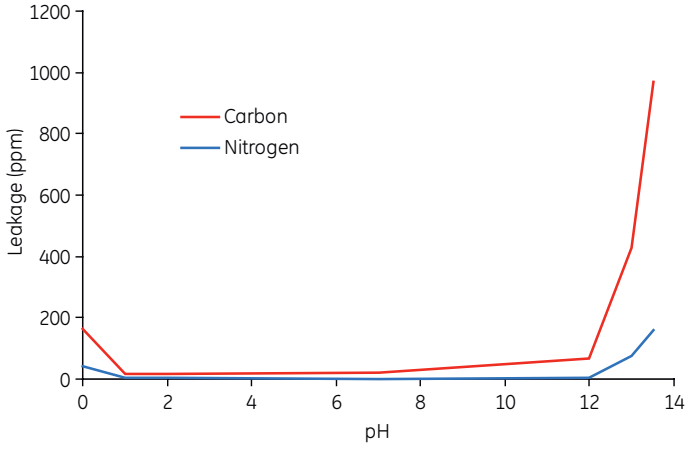


Fig 5. Stability of IgSelect at different pH.

Cleaning-in-place and sanitization

A cleaning or sanitization protocol has to be designed for each application. The resistance to cleaning with sodium hydroxide has been studied. Each blank purification cycle consisted of equilibration, loading, elution and cleaning-in-place (CIP) with 0.1 M NaOH + 1 M NaCl followed by re-generation. After every tenth cycle the dynamic binding capacity for IgG was determined. The capacity decreased as the number of CIP cycles increased, (Fig 6). We suggest regular cleaning with an acidic solution e.g. 0.1 M phosphoric acid or in combination with sodium chloride or ethanol, but with intermittent cycles consisting of 0.1 M NaOH.

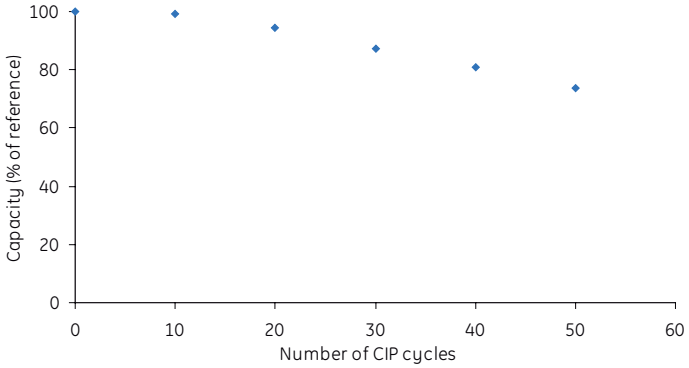


Fig 6. Cleaning in place with 0.1 M NaOH + 1 M NaCl. Contact time was 30 min per cycle.

Storage

It is recommended that the medium is stored in 20% ethanol at 4–8 °C. IgSelect is supplied, pre-swollen, in 20% ethanol.

Ordering information

Product[1]	Quantity	Code no.
IgSelect	25 ml	28-4113-01
IgSelect	1 l	28-4113-03
Prepacked HiTrap column	5 × 1 ml	28-4113-11
Prepacked HiTrap column	1 × 5 ml	28-4113-12

[1] IgSelect products are part of our Custom Designed Media program and are not yet standard products. If you are interested in large-scale quantities, please contact your local GE Healthcare representative.

For contact information for your local office, please visit, www.gelifesciences.com/contact

www.gelifesciences.com/bioprocess

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imagination at work

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