Affinity chromatography

Alpha-1 Antitrypsin Select

Alpha-1 Antitrypsin Select is a affinity chromatography resin with high selectivity for alpha-1 antitrypsin (AAT). The resin can be used for purifying human AAT from plasma (including Cohn fractions) as well as from recombinant or transgenic sources. In a first capture step, Alpha-1 Antitrypsin Select gives high purity and yield. The resin can also be used in intermediate purification/polishing to enhance product purity. Alpha-1 Antitrypsin Select is part of GE Healthcare's Custom Designed Media program (Fig 1).

Benefits of Alpha-1 Antitrypsin Select include:

- Efficient, industrial-scale purification of AAT by affinity chromatography
- High flow rates for processing of large sample volumes to increase throughput
- Reduced regulatory concerns (due to non-mammalian derived product) in the production of AAT for clinical applications
- Mild elution conditions at neutral pH

Resin characteristics

Alpha-1 Antitrypsin Select is based on porous, spherical agarose particles (the base matrix) with a covalently attached AAT binding protein (the ligand). The ligand is attached to the matrix via a long hydrophilic spacer arm to make it easily available for binding to the target molecule (Fig 2). The AAT affinity ligand was developed with technology from BAC BV (now part of Thermo Fisher Scientific Inc.). Ligand manufacturing, including fermentation and subsequent purification/formulation, is performed in the absence of mammalian components. The ligand itself was developed using Camelidae-derived, single-domain antibody fragments from the immune response of llamas towards the target human AAT molecule. The gene of the selected protein was cloned into a yeast cell expression system.

The characteristics of Alpha-1 Antitrypsin Select are summarized in Table 1.

Fig 1. Alpha-1 Antitrypsin Select chromatography resin is designed for affinity purification of AAT in industrial processes.

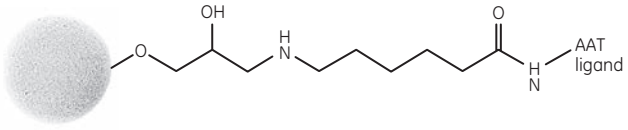


Fig 2. Structure of Alpha-1 Antitrypsin Select.

Table 1. Main characteristics of Alpha-1 Antitrypsin Select

Matrix	Highly cross-linked agarose, spherical
Particle size, d_{50v}*	75 µm
Ligand	Recombinant protein (M_r 13 025) produced in *Saccharomyces cerevisiae*
Ligand concentration	Approx. 5.5 mg/mL resin
Total binding capacity†	Approx. 10 mg AAT/mL resin
Flow velocity	Minimum 600 cm/h in a 1 m diameter column, with 20 cm bed height at 20°C using buffers with the same viscosity as water at < 0.3 MPa (3 bar)
pH stability, operational‡	3 to 10
pH stability, CIP§	2 to 11
Working temperature¶	4°C to 30°C

* Median particle size of the cumulative volume distribution.

† Protein in excess is loaded in PBS buffer, pH 7.4 on Tricorn™ 5/50 column. The binding capacity is obtained by measuring the amount of bound and eluted protein in 20 mM Tris, 2 M $MgCl_2$, pH 7.4.

‡ pH interval where the resin can be operated without significant change in function.

§ pH interval where the resin can be subjected to cleaning in place (CIP) or sanitization in place without significant change in function.

¶ Recommended long-term storage conditions: 2°C to 8°C, 20% ethanol.

Principles

Affinity chromatography is one of the chromatographic methods for purification of a specific molecule or a group of molecules from complex mixtures. The technique offers high selectivity and usually high capacity for the target molecule. As affinity chromatography is a binding technique, the sample volume does not affect the separation. Diluted samples can be applied, although capacity is commonly somewhat lower with more diluted sample. The immobilized ligand adsorbs the target molecule under suitable binding conditions. Under suitable elution conditions, the target molecule is desorbed. These conditions depend on the target molecule, feed composition, and the chromatography resin, and they must be evaluated together with other chromatographic parameters (e.g., sample load, flow velocity, bed height, regeneration, cleaning-in-place, etc.) to establish the conditions that will bind the largest amount of target molecule in the shortest time and with the highest product recovery. Regeneration should restore the original function of the resin. Depending on the nature of the sample, regeneration is normally performed after each cycle, followed by re-equilibration in start buffer. To prevent buildup of contaminants over time, more rigorous protocols may need to be applied (see Cleaning in place [CIP] and sanitization in place [SIP]).

Application

Application 1: typical capture protocol

A typical protocol for using Alpha-1 Antitrypsin Select to capture AAT from plasma is shown in Figure 3.

Chromatographic conditions

Sample:	100 mL plasma diluted 3-fold in 20 mM Tris, 150 mM NaCl, pH 7.4
Equilibration, loading, and wash buffer:	20 mM Tris, 150 mM NaCl, pH 7.4
Elution buffer:	20 mM Tris, 2 M $MgCl_2$, pH 7.4
Stripping buffer:	PBS, pH 2
Column:	8 mL of Alpha-1 Antitrypsin Select packed in a HR10/10 column

Running procedure

Flow rate:	Sample load and wash 2.3 mL/min (150 cm/h) Elution 4.6 mL/min (300 cm/h)
Equilibration:	10 CV binding buffer
Sample size:	100 mL prepared sample
Wash:	4 CV binding buffer
Elution 1:	4 CV elution buffer
Elution 2:	4 CV stripping buffer

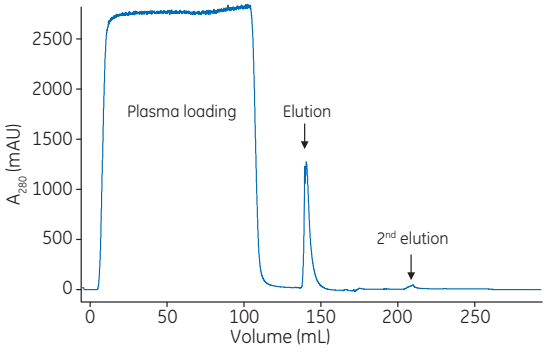
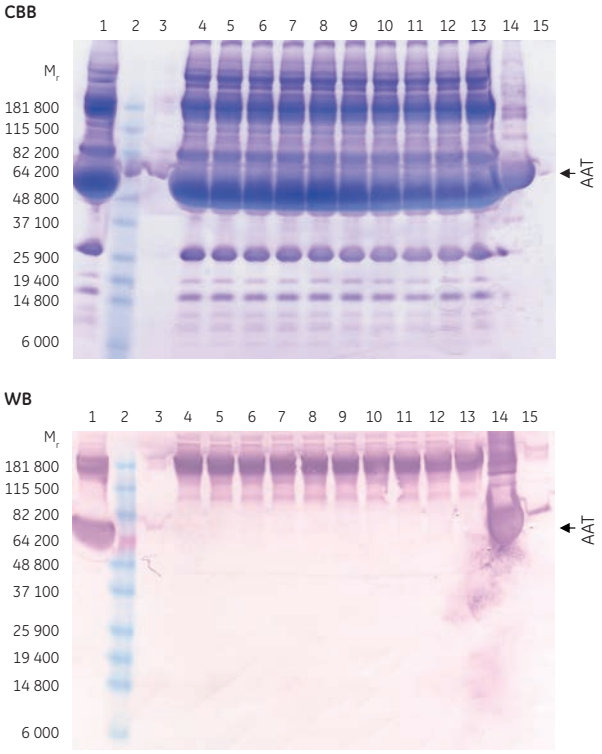


Fig 3. UV_{280} absorbance curve for plasma loading and elution of AAT using Alpha-1 Antitrypsin Select as the first capture step for purifying AAT from plasma. Including a wash step prior to the first elution would improve the purity of the eluted peak even further.

The protein content of the starting material (plasma), the flow-through fractions and the two elution fractions were analyzed by gel electrophoreses and visualized by Coomassie™ Brilliant Blue (CBB) staining and Western blot (WB) using a goat anti-human AAT antibody (Fig 4). Alpha-1 Antitrypsin Select depleted AAT from the plasma. AAT eluted in the first elution (lane 14). The extra signals visible in the WB are most probably IgG due to cross-reactivity of the used antibodies.



Lane 1	Start material	**9**	Flow through 7
2	Benchmark Prestained Marker	**10**	Flow through 8
3	Flow through 1	**11**	Flow through 9
4	Flow through 2	**12**	Flow through 10
5	Flow through 3	**13**	Flow through 11
6	Flow through 4	**14**	Elution 1
7	Flow through 5	**15**	Elution 2
8	Flow through 6		

Fig 4. CBB staining and WB of the start material (plasma), the non-retained fractions and the elution fractions. Lane 14 shows AAT from the first elution peak.

Application 2: polishing a registered AAT drug

This second example shows the polishing of a commercially-available AAT drug approved for infusion therapy.

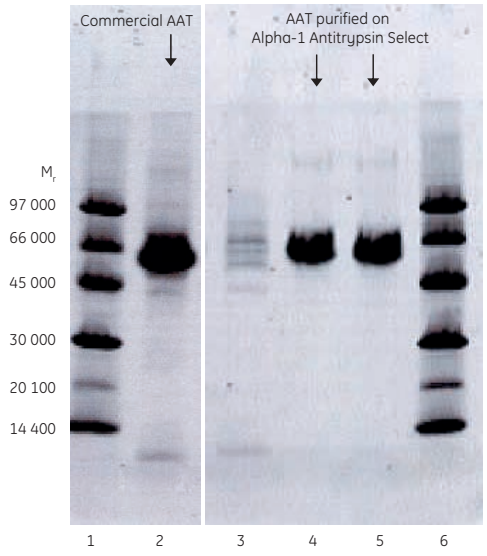
Chromatographic conditions

Sample:	A registered pharmaceutical AAT drug 1000 mg was diluted to 25 mg/mL using water for injection and thereafter further to 1.5 mg/mL using PBS buffer, pH 7.4.
Equilibration, loading, and washing buffer:	PBS buffer, pH 7.4
Elution buffer:	20 mM Tris, 2 M $MgCl_2$, pH 7.4
Column:	Tricorn 5/50 packed with 1 mL of Alpha-1 Antitrypsin Select

Running procedure

Flow rate:	Sample load 0.2 mL/min (61 cm/h) Wash and elution 0.5 mL/min (153 cm/h)
Equilibration:	10 CV binding buffer
Sample size:	Approx. 14 mL of prepared AAT sample
Wash:	15 CV binding buffer
Elution:	10 CV elution buffer

Gel electrophoreses was run on SDS PAGE Gradient 8–25 gels. The gels were stained with Deep Purple™ total protein stain and scanned in a Typhoon™ scanner. Figure 5 shows the results.



Lane **1** Protein LMW standards
2 Commercial AAT for infusion therapy
3 Flow-through fraction with impurities
4–5 Elution fraction of commercial AAT after purification on Alpha-1 Antitrypsin Select
6 Protein LMW standards

Fig 5. Gel electrophoreses of a commercially-available AAT drug before and after purification on Alpha-1 Antitrypsin Select.

Using Alpha-1 Antitrypsin Select in the chromatographic procedure described above improves the purity of a commercially-available AAT drug approved for infusion therapy.

Stability

The ligand is immobilized to the agarose base matrix via stable amide bonds that ensure high chemical stability and low leakage. Figure 6 shows the stability of Alpha-1 Antitrypsin Select after one week's storage at 40°C in different solutions of various pH. In addition, total AAT binding capacity was determined for resins exposed to pH 2, 10, 11 and 12 (Fig 7).

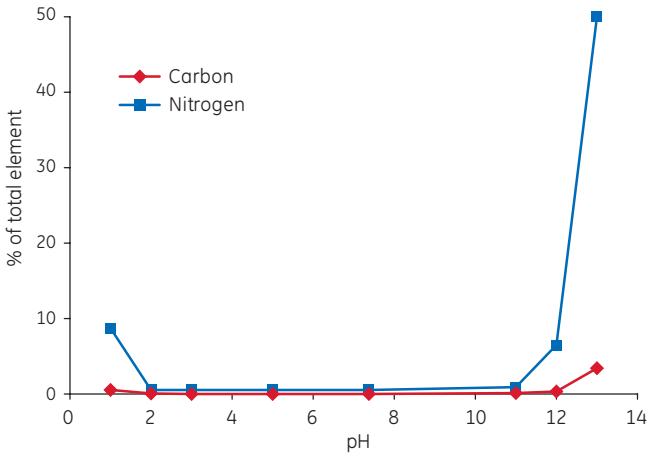


Fig 6. Relative loss of carbon and nitrogen during static incubation of Alpha-1 Antitrypsin Select at pH 1 to 13 at 40°C for seven days.

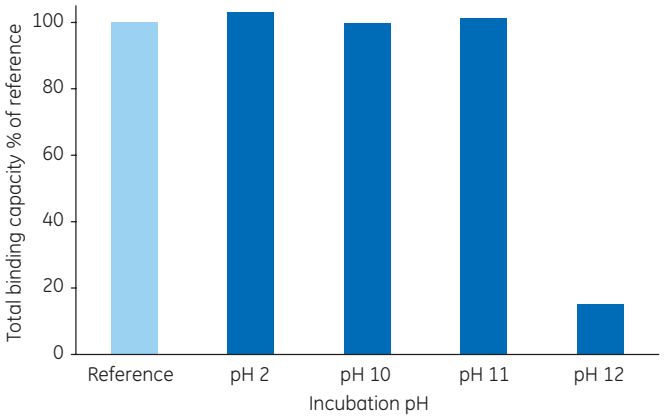


Fig 7. Total AAT binding capacity (determined as a percentage of reference) of Alpha-1 Antitrypsin Select after storage in solutions of different pH at 20°C for seven days.

The results demonstrate that Alpha-1 Antitrypsin Select has excellent chemical stability at pH 3–10. Significant loss of ligand functionality was not seen until pH 12, and loss of ligand itself was not severe until pH 13. The recommended pH range for short-term use, e.g. cleaning, is pH 2–11, while the recommended range for long-term use is pH 3–10.

For stability in commonly used cleaning and sanitization solutions, studies have been performed on KappaSelect and LambdaFabSelect resins, two other products employing Thermo Scientific™ CaptureSelect™ affinity ligands (Thermo Fisher Scientific). The results from these studies are expected to be valid for Alpha-1 Antitrypsin Select as well, except for stability at high pH, which is somewhat enhanced for LambdaFabSelect compared with other CaptureSelect ligand-containing products. For results, see data file for the respective product.

Leakage assay

For determination of ligand leakage from Alpha-1 Antitrypsin Select resin, the Thermo Scientific CaptureSelect Alpha-1 Antitrypsin Select Leakage ELISA Kit (Thermo Fisher Scientific) can be used.

Cleaning-in-place (CIP) and sanitization in place (SIP)

A cleaning or sanitization protocol should be designed for each application, as the efficiency of the protocol is strongly related to the feedstock and other related operating conditions. The recommended protocol comprises initial strip of the resin at low pH, and then subjecting the resin to NaOH of low concentration for cleaning. Lastly, PAB (120 mM phosphoric acid, 167 mM acetic acid, 2.2 % v/v benzyl alcohol) is used for final sanitization of the resin. PAB solution is sensitive to light and should be freshly made not to damage the resin. PAB solution should be stored in a dark

bottle and kept no longer than for a week. PAB solution has a pH of < 2, and resin stability can be limited in prolonged exposure at such a low pH.

1. 0.1 M citric acid, pH 2.1; 10 min; 13 CV
   10 CV PBS, pH 7.4
2. 10 mM NaOH, pH 12; 15 min; 19 CV
   10 CV PBS, pH 7.4
3. PAB; 15 min; 19 CV

Equilibrate the resin using equilibration buffer prior to next purification cycle.

Alpha-1 Antitrypsin Select was incubated at 20°C for 48 h in seven solutions commonly used for CIP. The total AAT binding capacity of the incubated resins was determined and compared with that of the untreated resin (Fig 8).

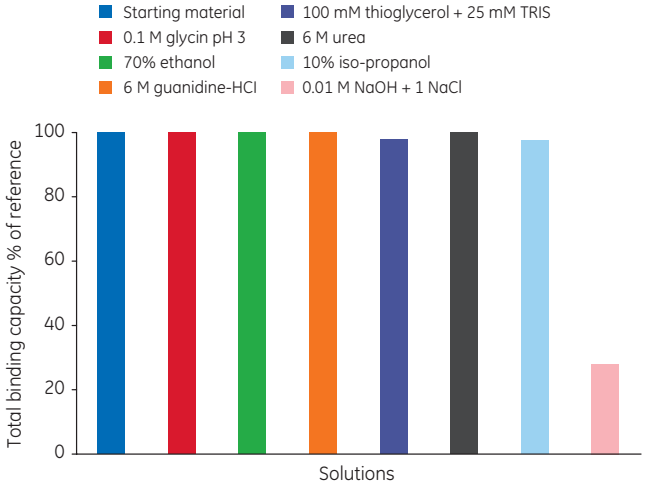


Fig 8. Total AAT binding capacity of Alpha-1 Antitrypsin Select (determined as a percentage of a reference) after storage in seven CIP solutions at 20°C for 48 h.

Results show that solutions such as 70% ethanol, guanidine hydrochloride, thioglycerol with Tris, urea, 0.1 M glycin pH 3 or 10% iso-propanol can be used to develop CIP protocols that will not decrease the binding capacity of Alpha-1 Antitrypsin Select. Basic conditions with pH in excess of pH 11 with or without added sodium chloride are not recommended as the ligand loses binding ability. For agarose matrices in general, avoid prolonged exposure (i.e., several days) to pH less than pH 2 as the agarose matrix slowly decomposes at low pH.

Storage

We recommend storage of Alpha-1 Antitrypsin Select resin in 20% ethanol at 2°C to 8°C. The resin is supplied as a suspension in 20% ethanol.

Ordering information

Product	Quantity	Product code
Alpha-1 Antitrypsin Select	25 mL	17547201
Alpha-1 Antitrypsin Select	200 mL	17547202
Alpha-1 Antitrypsin Select	1 L	17547203
Alpha-1 Antitrypsin Select	5 L	17547204

Related literature	Product code
Alpha-1 Antitrypsin Select Regulatory Support File	on request
Sofer, G. and Hagel, L. Cleaning, sanitization, and storage, in *Handbook of Process Chromatography: A Guide to Optimization, scale-up and validation.* Academic Press, Amsterdam, pp. 188–214 (1997)	18112156
Handbook: Affinity Chromatography, principles and methods	18102229