Alpha-1 Antitrypsin Select

AFFINITY CHROMATOGRAPHY RESIN

Alpha-1 Antitrypsin Select resin is a packed-bed affinity chromatography resin with high selectivity for alpha-1 antitrypsin (AAT). It 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 resin gives high purity and yield. The resin can also be used in intermediate purification/polishing to enhance product purity.

Alpha-1 Antitrypsin Select resin is especially attractive to biopharmaceutical manufacturers. Its key attributes include:

- Unique selectivity allows efficient industrial purification of AAT
- Outstanding pressure and flow rates reduce process times and improve process economy
- Mild elution conditions at neutral pH

Resin characteristics

Alpha-1 Antitrypsin Select resin is based on porous spherical agarose particles (the base matrix) with a covalently-attached AAT binding protein (the ligand). Figure 2 shows the partial structure of the resin.

The matrix used for Alpha-1 Antitrypsin Select resin is based on highly-rigid spherical agarose particles. Agarose is a natural polymer whose hydrophilic properties minimize structural changes of the target molecule as well as non-specific adsorption to the matrix. The rigid base matrix offers outstanding pressure and flow properties (Fig 3), which are one of the key requirements for cost-effective, large-scale use. Alpha-1 Antitrypsin Select resin permits a wide working range of flow velocities, bed heights, and sample viscosities, which helps minimize processing costs.

The manufacturing process of the AAT-binding ligand, 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 lamas towards the target human AAT molecule. The gene of the selected protein was cloned into a yeast cell expression system.

Fig 1. Alpha-1 Antitrypsin Select resin has high affinity for alpha-1 antitrypsin (AAT). Outstanding pressure and flow properties make it ideal for costeffective, large-scale processing.

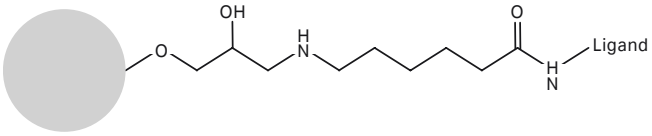


Fig 2. Partial structure of Alpha-1 Antitrypsin Select resin. The spacer arm between the base matrix and ligand facilitates effective binding of the target AAT molecule.

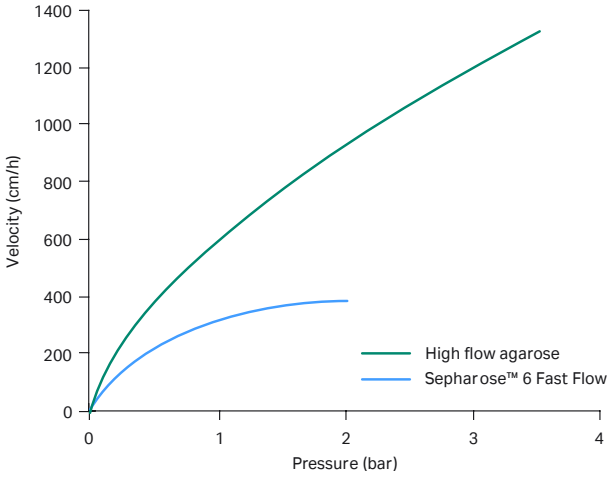


Fig 3. The high-flow agarose base matrix of Alpha-1 Antitrypsin Select resin displays superior open-bed pressure/flow properties compared to the base matrix of Sepharose™ 6 Fast Flow. Running conditions: BPG™ 300 column, open bed with water at 20°C at a settled bed height equal to 20 cm.

The AAT ligand is covalently bound to the agarose base matrix via a hydrophilic spacer to enhance accessibility and facilitate effective binding of the target molecule (see Fig 2). The ligand is attached via an amide linkage to give a chemically-stable covalent bound (see Figs 8 and 9).

Table 1 lists the key characteristics of Alpha-1 Antitrypsin Select resin.

Table 1. Characteristics of Alpha-1 Antitrypsin Select resin

Base matrix	**Highly cross-linked spherical agarose**
Average particle size[1]	75 µm (d_{50v})
Ligand	AAT binding ligand
Ligand density	Approx. 5.5 mg/mL resin
Binding capacity[2]	Approx. 10 mg AAT/mL resin
Storage buffer	20% ethanol in water
pH stability	
Short-term[3]	2 to 11
Long-term[4]	3 to 10

[1] d_{50v} is the median particle size of the cumulative volume distribution.
[2] Determined using a total capacity chromatography method.
[3] Short-term refers to the pH interval for regeneration and cleaning.
[4] Long-term refers to the pH interval where the resin is stable over a long period of time without adverse effects on the subsequent chromatographic performance.

Chromatography equipment

Alpha-1 Antitrypsin Select resin is well suited for use with most equipment commonly used for affinity chromatography from laboratory to production scale. Table 2 lists suitable laboratory and process columns from Cytiva.

Table 2. Suitable chromatography columns

Column family	**Range (bed diameter)**
Tricorn™	5 mm, 10 mm
HiScale™	16 to 50 mm
AxiChrom™	50 to 1000 mm

Principle

The general principle of affinity chromatography is that a biospecific ligand attached to a chromatographic base matrix is exposed to its corresponding adsorbent under conditions that favor specific binding. The target adsorbent thus binds specifically and reversibly to the ligand. Unbound substances are washed away, after which the target substance is recovered by applying conditions that favor its elution. Binding also concentrates samples so that the target protein is collected in a purified and concentrated form. Figure 4 illustrates this principle.

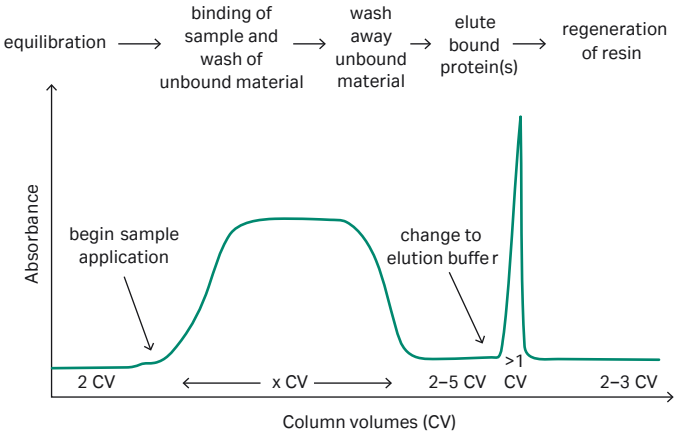


Fig 4. A typical affinity purification. The target protein is usually eluted in a purified and concentrated form.

The binding and elution conditions used in an affinity purification depend on the target molecule, feed composition and the chromatography resin. These must be studied together with other chromatographic parameters such as sample load, flow velocity, bed height, regeneration and cleaning-in-place to establish the optimal chromatographic conditions for each specific purification.

Application 1: typical capture protocol

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

Chromatographic conditions

Sample:	100 mL plasma diluted 3-fold in 20 mM Tris, 150 mM NaCl, pH 7.4
Binding 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 resin 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

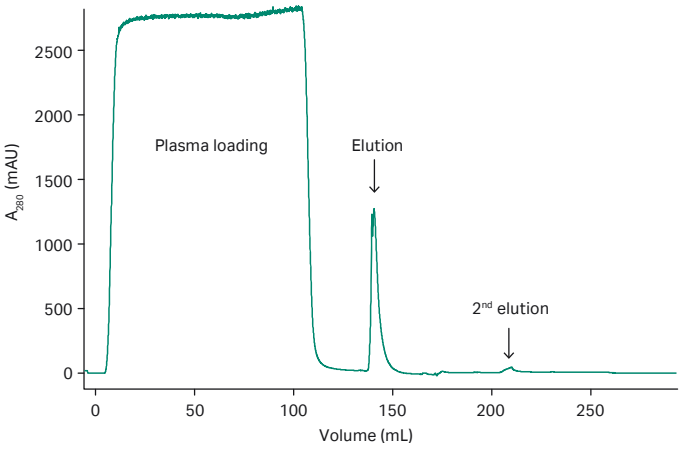
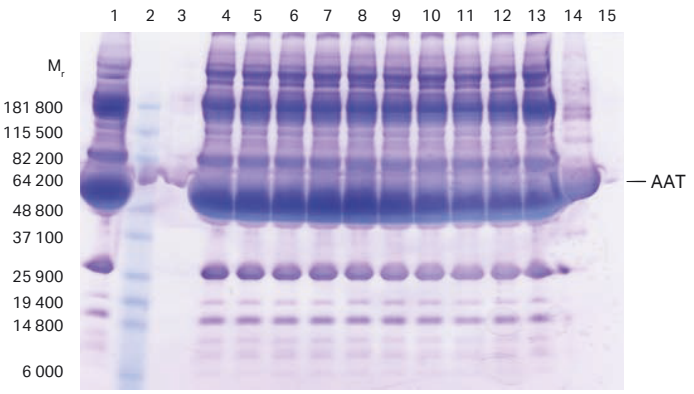


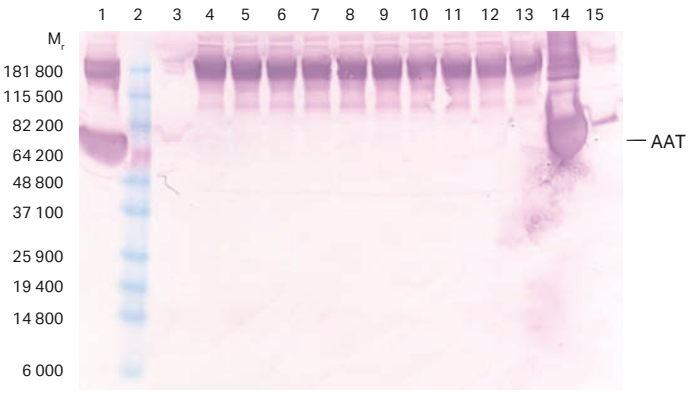
Fig 5. UV_{280} absorbance curve for plasma loading and elution of AAT using Alpha-1 Antitrypsin Select resin as the first capture step for purifying AAT from plasma. Including a wash step prior to the first elution would improve the purity of the eluted peak even further.

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 6). Alpha-1 Antitrypsin Select depleted AAT from the plasma. AAT then 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.

CBB



WB



Lanes

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

Fig 6. 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.

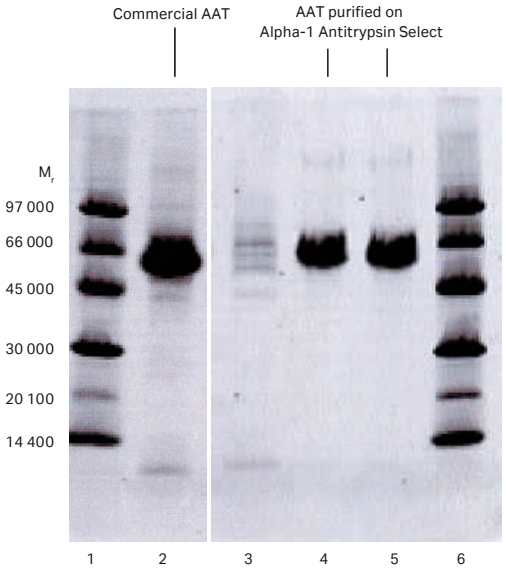
Gel electrophoreses was run on SDS PAGE Gradient 8–25 gels. The gels were stained with Deep Purple total protein stain and scanned in a Typhoon™ scanner. Figure 7 shows the results.

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.
Binding 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 resin

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



Lanes

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 resin
6. Protein LMW standards

Fig 7. Gel electrophoreses of a commercially-available AAT drug before and after purification on Alpha-1 Antitrypsin Select resin.

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

Stability

The Alpha-1 Antitrypsin Select ligand is immobilized to the agarose base matrix via stable amide bonds that ensure high chemical stability and low ligand leakage.

Chemical stability was investigated in static soak experiments in solutions of pH 1, 2, 3, 7.4, 11, 12 and 13 for seven days at 20°C. After incubation, the supernatants were analyzed for total organic carbon and total nitrogen. Released nitrogen represents ligand, since nitrogen occurs only in the ligand and in the ligand coupling spacer arm. Released carbon may originate either from the ligand or the agarose base matrix.

Relative loss of carbon and nitrogen was calculated based on their total amounts (Fig 8). In addition, total AAT binding capacity was determined for resins exposed to pH 2, 10, 11 and 12 (Fig 9).

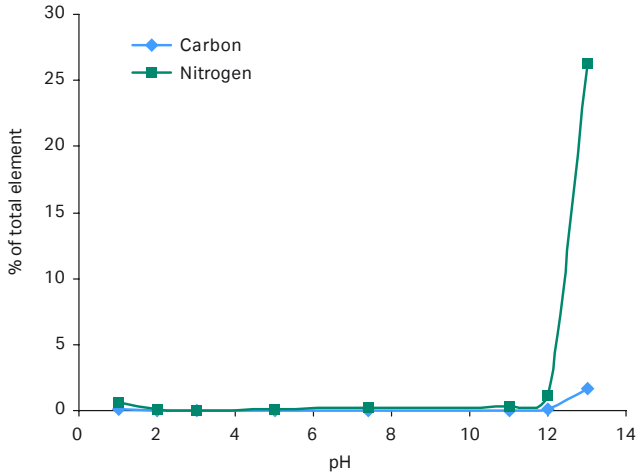


Fig 8. Relative loss of carbon and nitrogen during static incubation of Alpha-1 Antitrypsin Select at pH 1 to 13 at 20°C for seven days.

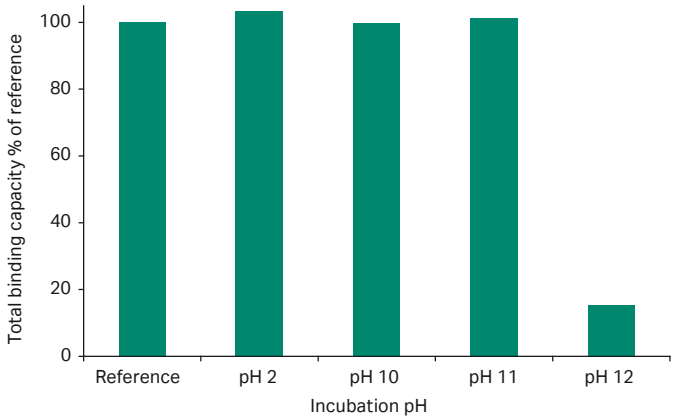


Fig 9. Total AAT binding capacity (determined as a percentage of reference) of Alpha-1 Antitrypsin Select after storage in solutions of different pH at 20°C for seven days.

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.

Leakage assay

An assay for determining ligand leakage is available from Thermo Fisher Scientific via their website (www.thermofisher.com)

Cleaning-in-place (CIP) and sanitization

Alpha-1 Antitrypsin Select resin was incubated at 20°C for 48 h in seven solutions commonly used for CIP. The total AAT binding capacity of the incubated media was determined and compared with that of the untreated medium (Fig 10).

Results show that solutions such as 70% ethanol, guanidine hydrochloride, thioglycerol with Tris, urea 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 since the ligand looses 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.

Note that a cleaning or sanitization protocol must be designed for each specific application.

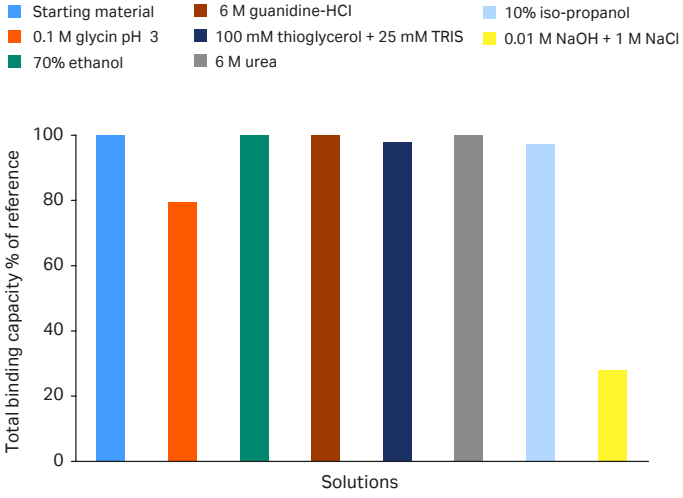


Fig 10. Total AAT binding capacity of Alpha-1 Antitrypsin Select (determined as a percentage of a reference) after storage in seven CIP solutions at 20°C for 48 h.

Storage

Store unused resins at 4°C to 8°C in 20% ethanol. Alpha-1 Antitrypsin Select resin is supplied in 20% ethanol.

Ordering information

Product[1]	Size[2]	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

[1] Part of the Custom Design Media program at Cytiva.
[2] Larger quantities are available. Please contact your local Cytiva representative.

cytiva.com

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