VIISelect

AFFINITY CHROMATOGRAPHY

VIISelect is an affinity resin designed for selective binding of coagulation Factor VII. It can be used, under mild elution conditions, for purifying Factor VII from plasma as well as from recombinant sources. VIISelect is part of Cytiva's Custom Designed Media program.

Benefits of VIISelect include:

- Efficient, industrial-scale purification of Factor VII by affinity chromatography
- High flow rates and processing of large sample volumes for increased throughput
- Reduced regulatory concerns (due to non-mammalian derived product) in the production of Factor VII for clinical applications

Efficient processes for the purification of recombinant blood coagulation factors are needed in the development of treatments for hemophilia patients. VIISelect is an affinity resin designed for the purification of Factor VII/VIIa, a blood factor used for treatment of patients with FVII deficiency as well as patients with hemophilia A and B with inhibitors to FVIII or FIX.

Medium characteristics

VIISelect is based on porous, spherical agarose particles (the base matrix) with a covalently attached Factor VII 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 1). The Factor VII 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 Factor VII molecule. The gene of the selected protein was cloned into a yeast cell expression system.

The characteristics of VIISelect are summarized in Table 1.

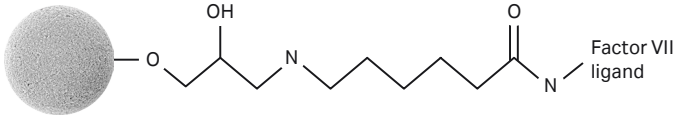


Fig 1. Partial structure of VIISelect.

Table 1. Main characteristics of VIISelect

Matrix	Highly cross-linked agarose, spherical
Particle size, d_{50V}*	75 µm
Ligand	Recombinant protein (M_r 14 080) produced in *Saccharomyces cerevisiae*
Ligand concentration	Approx. 6 mg/mL of resin
Total binding capacity†	Approx. 8 mg Factor VII/mL of resin
Flow velocity	At least 600 cm/h in a 1 m 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 12
Working temperature¶	4°C to 30°C

* Median particle size of the cumulative volume distribution.
† Protein in excess is loaded in 50 mM Tris, 150 mM NaCl, pH 7.5 on Tricorn™ 5/20 column. The binding capacity is obtained by measuring the amount of bound and eluted protein in 50 mM Tris, 1.5 M NaCl, 50% (v/v) propylene glycol, pH 7.5.
‡ pH interval where the medium can be operated without significant change in function.
§ pH interval where the medium 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. In order to prevent build-up of contaminants over time, more rigorous protocols may have to be applied (see Cleaning-in-place).

Application: purifying Factor VII spiked in human plasma

This example shows the purification of Factor VII from a commercially-available drug approved for infusion therapy spiked in human plasma. Note that the experimental conditions are not optimized.

Chromatographic conditions

Sample:	A registered pharmaceutical Factor VII drug was diluted with solution for injection and spiked in human plasma.
Equilibration, loading, and wash buffer:	50 mM Tris, 150 mM NaCl, pH 7.5
Elution buffer:	50 mM Tris, 1.5 M NaCl, 50% (v/v) propylene glycol, pH 7.5
Column:	Tricorn 5/20 packed with 0.45 mL of VIISelect

Run procedure

Flow rate:	Sample load 0.2 mL/min (61 cm/h) Wash and elution 0.5 mL/min (153 cm/h)
Equilibration:	10 column volumes (CV) binding/equilibration buffer
Loaded amount:	7 mg/mL resin (approx 88% of the maximum capacity)
Wash:	12 CV binding/equilibration buffer
Elution:	12 CV elution buffer

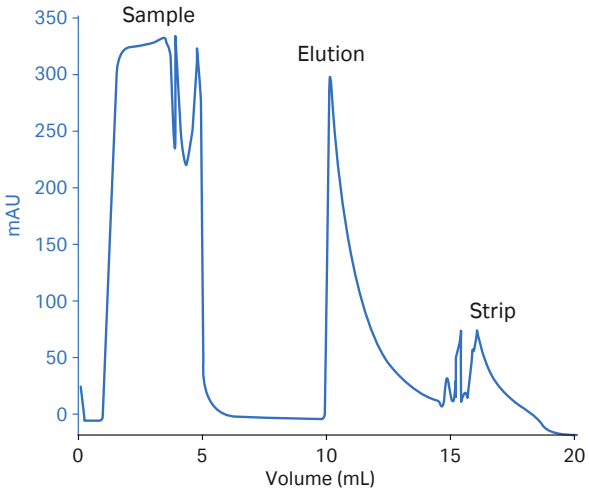
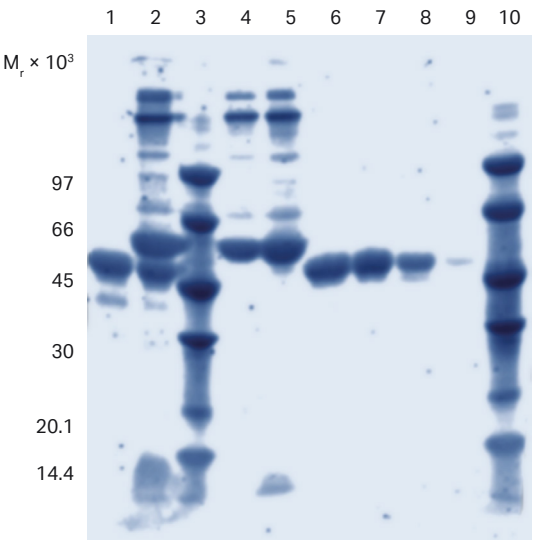


Fig 2. UV_{280} absorbance curve for plasma loading and elution of Factor VII using VIISelect as the first capture step for purifying Factor VII from plasma spiked with a commercially-available Factor VII drug. Note that the experimental conditions are not optimized and in this experiment the loaded amount was below the maximum capacity of VIISelect.

Gel electrophoreses was run on SDS PAGE gradient gel 8%–16% under non-reducing conditions. The gels were stained with Deep Purple total protein stain and scanned in a Typhoon™ scanner. Figure 3 shows the results.



Lanes

1. Commercial Factor VII
2. Commercial Factor VII spiked in plasma
3. LMW Marker
4. Flow-through fraction with impurities
5. Wash after sample loading
6. Elution fraction 5 after purification on VIISelect
7. Elution fraction 6 after purification on VIISelect
8. Elution fraction 1-12 (pooled) after purification on VIISelect
9. Strip after elution
10. LMW Marker

Fig 3. Gel electrophoreses of a commercially-available Factor VII drug before and after purification on VIISelect.

Stability

The ligand is immobilized to the agarose base matrix via stable amide bonds that ensure high chemical stability and low leakage. Figure 4 shows the stability of VIISelect after storage in different solutions of various pH at 40°C for one week. Ligand leakage is low in the pH range 2 to 12. At pH values > 12, both carbon and nitrogen are released, which indicates hydrolysis of the ligand.

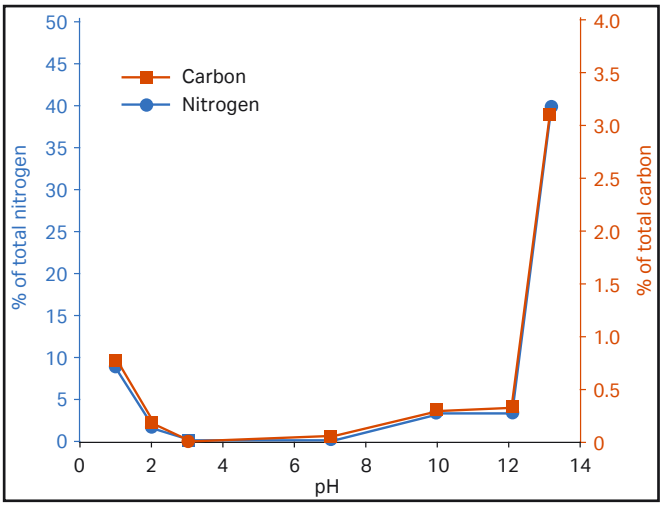


Fig 4. Stability of VIISelect at different pH values.

For stability in commonly used CIP and sanitization solutions, studies have been performed on KappaSelect and LambdaFabSelect resin, two other products employing Thermo Scientific™ CaptureSelect™ affinity ligands (Thermo Fisher Scientific). The results from these studies are expected to be valid for VIISelect 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 VIISelect medium, the Thermo Scientific CaptureSelect VIISelect 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.

Storage

The recommended storage conditions are 20% ethanol at 2°C to 8°C. VIISelect is supplied as a suspension in 20% ethanol.

Ordering information

Product	Quantity	Product code
VIISelect	25 mL	17547701
VIISelect	200 mL	17547702
VIISelect	1 L	17547703
VIISelect	5 L	17547704

Related literature	
VIISelect Regulatory Support File	on request
Affinity Chromatography: Principles and Methods, Handbook	18102229
Affinity Columns and Media, Selection guide	18112186

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