GE Healthcare
Life Sciences

VIISelect

VIISelect is an affinity medium 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 GE Healthcare's Custom Designed Media program.

VIISelect affinity chromatography medium supports:

- 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 medium designed for the purification of Factor VII/VIIa, a blood factor used for treatment of hemophilia patients who have developed inhibitors against replacement coagulation factors.

Characteristics of the medium

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 manufacturing process for the Factor VII 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 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.

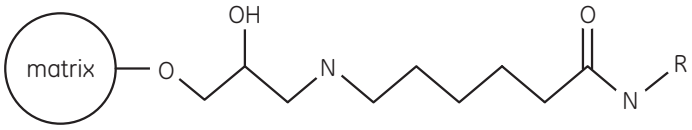


Fig 1. Partial structure of VIISelect. "R" stands for the Factor VII ligand.

Table 1. Main characteristics of VIISelect

Matrix	Highly cross-linked high-flow agarose
Particle size*	75 µm (d_{50v})
Ligand	Recombinant protein (M_r 14 080) produced in *Saccharomyces cerevisiae*
Ligand density	Approx. 5.7 mg/mL of medium
Binding capacity	Approx. 8 mg/mL of medium†
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 < 3 bar (0.3 MPa)
pH stability	
long term	3–10
short term	2–12
Working temperature‡	4°C to 30°C

* d_{50v} is the mean particle size of the cumulative volume distribution
† Determined using a static capacity chromatography method
‡ Recommended long-term storage conditions: 2°C to 8°C, 20% ethanol

Principles

Affinity chromatography separates proteins on the basis of a reversible interaction between a protein (or group of proteins) and a specific ligand coupled to a chromatography base matrix. The technique offers high selectivity and usually high capacity for the protein(s) of interest. The immobilized ligand adsorbs the protein(s) under suitable binding conditions and desorbs them during suitable elution conditions. These conditions depend on the target molecule, feed composition, and the chromatography medium, 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 medium. 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.
Binding/equilibration 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 medium (below the maximum capacity)
Wash:	12 CV binding/equilibration buffer
Elution:	12 CV elution buffer

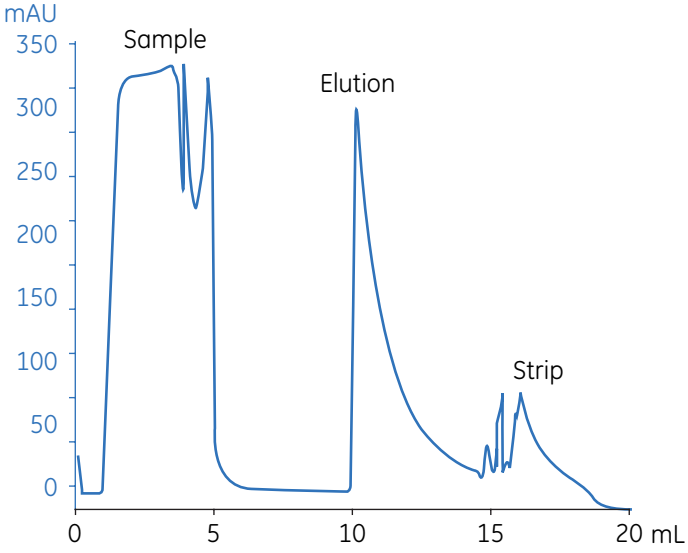
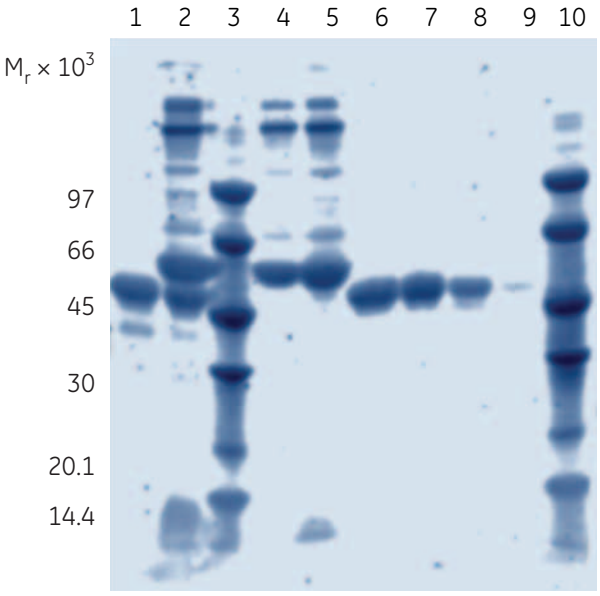


Fig 2. UV_{280} absorbance curve for plasma loading and elution of Factor VII using VIISelect as the first capture step for purifying Factor VII from plasma spiked with a commercially-available Factor VII drug. Note that the experimental conditions are not optimized and in this experiment the loaded 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. Low Molecular Weight 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. Low Molecular Weight 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 20°C for one week. Ligand leakage is low in the pH range 2 to 12 (one week at 20°C). At pH values > 12, both carbon and nitrogen are released, which indicates hydrolysis of the ligand.

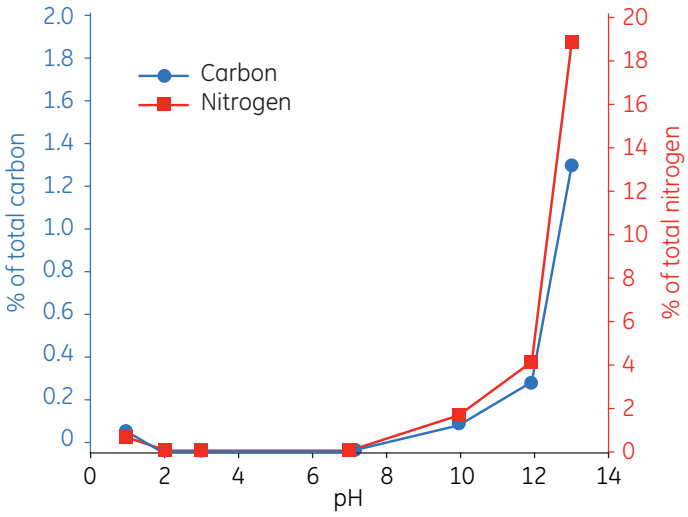


Fig 4. Stability of VIISelect at different pH values.

Leakage assay

An assay for determination of ligand leakage is available from BAC BV (Bio Affinity Company, The Netherlands) through their website (www.captureselect.com).

Cleaning-in-place

A cleaning protocol for VIISelect may consist of 20 mM sodium hydroxide or a combination of an acidic solution, for example 0.2 M citric acid (pH 2) or 0.2 M glycine (pH 2.5), and 20 mM sodium hydroxide. However, prolonged exposure (i.e., several days) to pH < 2 should be avoided due to slow decomposition of the agarose matrix at low pH. A pH > 12 should be avoided due to limited ligand stability under alkaline conditions. A cleaning or sanitization protocol has to be designed for each application.

Storage

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

Ordering information

Product	Quantity	Code no.
VIISelect	25 mL	17-5477-01
VIISelect	200 mL	17-5477-02
VIISelect	1 L	17-5477-03
VIISelect	5 L	17-5477-04

Related literature	Code no.
VIISelect Regulatory Support File	on request
Affinity Chromatography: Principles and Methods, Handbook	18-1022-29
Affinity Columns and Media, Selection Guide	18-1121-86

For local office contact information, visit
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