VIIISelect

AFFINITY CHROMATOGRAPHY

VIIISelect is an affinity chromatography resin designed for the purification of recombinant β domain-depleted factor VIII (FVIII) under mild elution conditions. VIIISelect is part of Cytiva Custom Designed Media program.

Benefits of VIIISelect include:

- Efficient industrial-scale purification of recombinant factor VIII, with high yields and retained specific activity
- High selectivity and excellent scalability
- Reduced regulatory concerns (due to non-mammalian-derived product) in the production of FVIII for clinical applications

Efficient purification processes of recombinant blood coagulation factors are needed for treating hemophilia patients. VIIISelect is an affinity chromatography resin designed for the purification of recombinant factor VIII, a key recombinant blood factor used for the treatment of Hemophilia A. Due to the sensitive nature of the factor VIII molecule, it is important to limit the number of steps in the downstream process. The high selectivity and yield obtained using VIIISelect enables a robust and efficient purification process with excellent purity obtained in one step. Low ligand leakage is an additional property that makes this resin highly suitable for large-scale production of recombinant FVIII.

Resin characteristics

VIIISelect is based on porous, spherical agarose particles (the base matrix) with a covalently attached FVIII 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 FVIII 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 FVIII molecule. The gene of the selected protein was cloned into a yeast cell expression system. The characteristics of VIIISelect are summarized in Table 1.

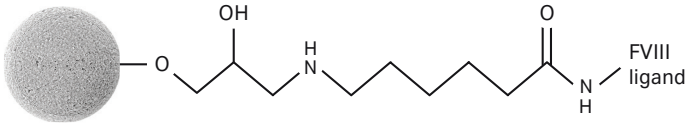

Fig 1. Partial structure of VIIISelect.

Table 1. Main characteristics of VIIISelect

Matrix	Highly cross-linked agarose, spherical
Particle size, d_{50v}*	75 µm
Ligand	Recombinant protein (M_r 13 000) produced in *Saccharomyces cerevisiae*
Ligand concentration	Approx. 0,7 mg/mL of resin
Dynamic binding capacity†	Approx. 20 000 IU FVIII/mL of resin
Flow velocity	Up to 300 cm/h at 30 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.
† As determined by McCue *et al.* (see Further reading)
‡ 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. In order 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]).

Binding and elution conditions

A typical protocol for using VIIISelect, with recommended buffers, is described below:

Equilibration/loading buffer:	10 mM histidine, 20 mM calcium chloride, 300 mM sodium chloride, and 0.02% Tween™ 80 at pH 7.0
Wash buffer 1:	20 mM histidine, 20 mM calcium chloride, 300 mM sodium chloride, and 0.02% Tween 80 at pH 6.5
Wash buffer 2:	20 mM histidine, 20 mM calcium chloride, 1.0 M sodium chloride, and 0.02% Tween 80 at pH 6.5.
Elution buffer:	20 mM histidine, 20 mM calcium chloride, 1.5 M sodium chloride, and 0.02% Tween 80 dissolved in 50% ethylene glycol at pH 6.5

1. Pack the column with VIIISelect.
2. Equilibrate with 10 CV (column volumes) of equilibration buffer.
3. Load the sample in loading buffer. Recombinant factor VIII can be applied directly to the VIIISelect column from clarified cell lysates or supernatants.
4. Wash with 5 CV of washing buffer 1.
5. Wash with 5 CV of washing buffer 2.
6. Elute with 5-10 CV of elution buffer.
7. Regenerate the column with regeneration buffer
8. Perform CIP

Regeneration restores the function of the resin.

Buffers should always contain Ca^{2+} ions in order to promote formation of the active conformation of factor VIII. The presence of a surfactant is needed to inhibit surface-induced denaturation. Neutral pH buffers and histidine should always be used for binding, washing, and elution for maintaining the specific factor VIII activity. Depending on the nature of the applied material to VIIISelect, regeneration is normally needed after each cycle, followed by re-equilibration in equilibration/loading buffer.

Stability

The ligand is immobilized to the agarose base matrix via stable amide bonds that ensure high chemical stability and low leakage. Figure 2 shows the stability of VIIISelect after one week's storage at 40°C in different solutions of various pH values. At pH values above 12, both carbon and nitrogen are released, which indicates hydrolysis of the ligand. Leakage of carbon and nitrogen is low in the pH range 2 to 10.

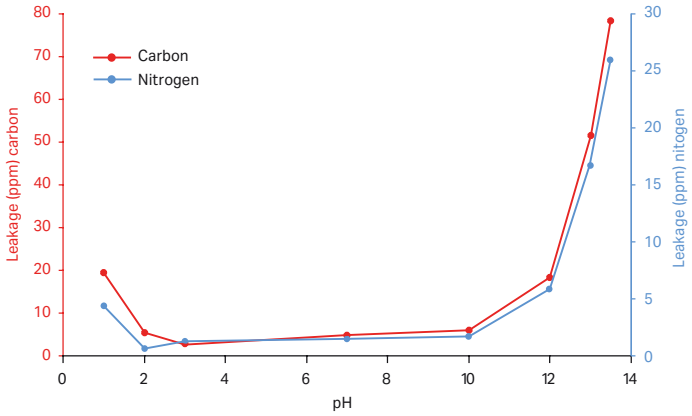

Fig 2. Stability of VIIISelect at different pH.

These results were confirmed via measuring the ligand content after storage at different pH values.

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

We recommend storage of VIIISelect resin in 20% ethanol at 2°C to 8°C. The resin is supplied as a suspension in 20% ethanol.

Further reading

McCue, J.T., Selvitelli, K., Walker, J. Application of a novel affinity adsorbent for the capture and purification of recombinant Factor VIII compounds. *Journal of Chromatography A* **1216**, 7824–7830 (2009).

Ordering information

Product	Quantity	Product code
VIIISelect	5 l	17545004
VIIISelect	500 ml	17545002
VIIISelect	25 ml	17545001

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

cytiva.com/bioprocess

Cytiva and the Drop logo are trademarks of Global Life Sciences IP Holdco LLC or an affiliate.

Thermo Scientific and CaptureSelect are trademarks of Thermo Fisher Scientific Inc. and its subsidiaries. Tween is a trademark of Uniqema Americas LLC. All other third-party trademarks are the property of their respective owners.

VIIISelect, KappaSelect, and LambdaFabSelect incorporates Thermo Fisher Scientific's proprietary ligand technology, which has been exclusively licensed to Cytiva for use in chromatography separation.

© 2020 Cytiva

All goods and services are sold subject to the terms and conditions of sale of the supplying company operating within the Cytiva business. A copy of those terms and conditions is available on request. Contact your local Cytiva representative for the most current information.

For local office contact information, visit cytiva.com/contact

CY13474-23Jul20-DF