

VIIISelect

VIIISelect is an affinity chromatography medium (resin) designed for the purification of recombinant β domain-depleted factor VIII.

Key characteristics of VIIISelect include:

- Efficient purification of recombinant β -depleted factor VIII, with high yields and retained specific activity
- High selectivity
- Excellent scalability
- Animal-free production

Efficient purification processes of recombinant blood coagulation factors are needed for treating hemophilia patients. VIIISelect is an affinity chromatography medium designed for the purification of recombinant β domain-depleted 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. Animal-free production and low ligand leakage are additional properties that make this medium highly suitable for large-scale production of recombinant β domain-depleted factor VIII. VIIISelect is part of GE Healthcare's Custom Designed Media program.

Medium characteristics

VIIISelect is based on highly cross-linked agarose base matrix, which enables rapid processing of large sample volumes. The ligand, a 13 kD recombinant protein, is attached to the porous base matrix via a hydrophilic spacer arm making it easily available for binding to recombinant β domain-depleted factor VIII (Fig 1). Table 1 summarizes the main characteristics of VIIISelect.

Functional principles

Affinity chromatography exploits an immobilized ligand that adsorbs a specific molecule or group of molecules under suitable binding conditions and desorbs them under suitable elution conditions. These conditions depend on the target molecule, feed composition, and chromatography medium, and must be studied together with other chromatographic parameters (e.g., sample load, flow velocity, bed height,

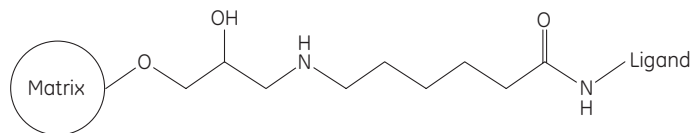


Fig 1. Partial structure of VIIISelect.

regeneration, and cleaning-in-place) to establish the conditions that will bind the target molecule with the highest product recovery.

Recombinant factor VIII can be applied directly to the VIIISelect column from clarified cell lysates or supernatants.

Table 1. Main characteristics of VIIISelect

Matrix	highly cross-linked agarose
Average particle size	75 μ m
Ligand	Recombinant protein (M_r 13 000) produced in <i>S. cerevisiae</i> .
Capacity	Typically 20 000 IU/ml gel
Recommended flow rate	Up to 300 cm/h at 30 cm bed height
Maximum back pressure	0.3 MPa, 3 bar
pH stability	
Long term	3–10
Short term	2–12

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
Washing buffer 1:	20 mM histidine, 20 mM calcium chloride, 300 mM sodium chloride, and 0.02% Tween 80 at pH 6.5
Washing 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.
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.



Buffers should always contain Ca²⁺ 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 linked to the highly cross-linked base matrix via a stable amide bond. Figure 2 shows a study where VIIISelect was stored at room temperature at different pH values for one week. The figure shows that the stability is high between pH 3 and 10. We recommend long term storage between pH 3 and 10, and short term storage pH 2 and 12.

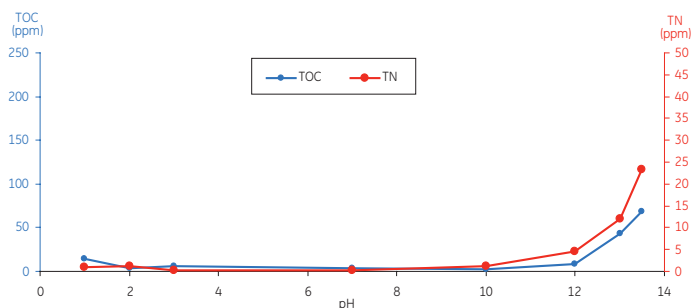


Fig 2. Stability of VIIISelect at different pH values, when stored for one week, TOC (Total Organic Carbon) and TN (Total Nitrogen).

At higher pH (> 12), there was leakage of both carbon and nitrogen, indicating loss of the ligand or parts of the ligand. These results were confirmed via a capacity study as well as measuring the ligand content after storage at different pH values.

For local office contact information, visit www.gelifesciences.com/contact

www.gelifesciences.com/bioprocess

GE Healthcare Bio-Sciences AB
Björkgatan 30
751 84 Uppsala
Sweden



Leakage assay

An assay for determining ligand leakage is available from BAC BV through their website: www.captureselect.nl/products

Storage

The recommended storage conditions are 20% ethanol at 4°C to 8°C. VIIISelect is supplied preswollen in a 20% ethanol solution.

Cleaning-in-place

A cleaning protocol for VIIISelect may consist of 0.1 M citric acid or 0.5 M phosphoric acid. However, prolonged exposure to pH < 2 should be avoided due to decomposition of the agarose base matrix. Sodium hydroxide (0.01 M) can be used alone or in combination with sodium sulfate/chloride as stabilizer. A cleaning and regeneration protocol must be designed for each application.

Ordering information

Product	Quantity	Code no.
VIIISelect	5 l	17-5450-04
VIIISelect	500 ml	17-5450-02
VIIISelect	25 ml	17-5450-01

This product is part of our Custom Designed Media program. If you are interested in large-scale quantities, please contact your local GE Healthcare representative.

Related literature

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

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GE Healthcare UK Limited
Amersham Place, Little Chalfont, Buckinghamshire, HP7 9NA, UK

GE Healthcare Europe, GmbH
Munzinger Strasse 5, D-79111 Freiburg, Germany

GE Healthcare Bio-Sciences Corp.
800 Centennial Avenue, P.O. Box 1327, Piscataway, NJ 08855-1327, USA

GE Healthcare Bio-Sciences KK
Sanken Bldg., 3-25-1, Hyakunincho, Shinjuku-ku, Tokyo 169-0073, Japan