

VIIISelect

VIIISelect is an affinity medium 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 activity.
- Highly Selective resin
- Excellent scalability
- Animal free production

Efficient purification processes of recombinant blood coagulation factors is needed for treatments of hemophilia patients. VIIISelect is an affinity resin designed for the purification of recombinant β domain depleted factor VIII, a key recombinant blood factor used for 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 yields obtained using VIIISelect enable a robust and efficient purification process with excellent purity obtained in one step. Animal free production and low ligand leakage are additional properties making this resin highly suitable for use in large scale production of recombinant β domain depleted factor VIII.

Medium characteristics

VIIISelect is based on Capto™ XP 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 to make it easily available for binding of recombinant β 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

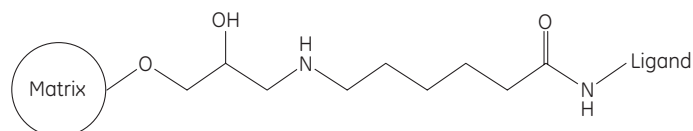


Fig 1. Partial structure of VIIISelect.

Table 1. Main characteristics of VIIISelect

| | |
|-----------------------|--|
| Matrix | Capto XP |
| Average particle size | 74 μ m |
| Ligand | 13 kD recombinant protein 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 |

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

Recombinant VIII can be applied directly to the VIIISelect column from clarified cell lysates or supernatants. Typical equilibration and washing buffer is ammonium acetate buffer at neutral pH in combination with NaCl, CaCl₂ and a surfactant (Tween or Triton).

Typical elution buffers consists of ethylenglycol, CaCl₂, histidine, NaCl and a surfactant at neutral pH.

Buffers should always contain Ca²⁺ ions in order to promote the formation of the active conformation of VIII. Presence of a surfactant is usually needed to inhibit surface induced denaturation.

Neutral pH buffers should always be used for binding, washing and elution in order to keep factor VIII active.



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. To prevent build up of contaminants over time, more rigorous protocols may have to be applied (see Cleaning-in-place and sanitization).

Stability

The ligand is linked to the Cpto XP base matrix via a stable amide bond. In a study where VIIISelect was stored at room temperature at different pH values for one week it was shown that the leakage is low between pH 2 and 12 (Fig 2). At higher pH there is a leakage of both carbon and nitrogen, indicating hydrolysis of the ligand.

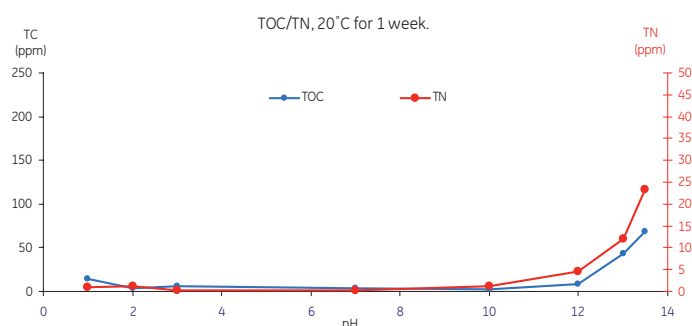


Fig 2. Stability of VIIISelect at different pH.

Cleaning-in-place and sanitization

A cleaning or sanitization protocol has to be designed for each application. A suggested cleaning protocol is to use a solution of low pH, for example 0.1 M acetic acid, alone or

in combination with sodium chloride or ethanol. Alternative cleaning -in-place protocols include 6 M guanidine hydrochloride or use of organic solvents such as 70 % ethanol. Sodium hydroxide should be used with care due to the limited stability under alkaline conditions.

Storage

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

Licensed use

VIIISelect incorporates BAC BV's proprietary ligand technology, which has been exclusively licensed to GE Healthcare in the field of purification of β domain depleted recombinant factor VIII. Other use of this product may require a separate license from BAC BV, Huizerstraatweg 28, 1411 GP Naarden, The Netherlands.

Ordering information

| Product | Quantity | Code no. |
|------------|----------|------------|
| 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.

Literature

| | |
|---|------------|
| Affinity Chromatography Handbook | 18-1022-29 |
| Affinity Columns and Media, Selection Guide | 18-1121-86 |

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

www.gelifesciences.com/bioprocess
www.gelifesciences.com

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

GE, imagination at work and GE Monogram are trademarks of General Electric Company.

Cpto is a trademark of GE Healthcare Companies.

All third party trademarks are the property of their respective owners.

© 2008 General Electric Company – All rights reserved.

First published February 2008.

All goods and services are sold subject to the terms and conditions of sale of the company within GE Healthcare which supplies them. A copy of these terms and conditions is available on request. Contact your local GE Healthcare representative for the most current information.

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

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

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

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

