IXSelect

AFFINITY CHROMATOGRAPHY

IXSelect is an affinity chromatography resin designed for selective binding of coagulation Factor IX (FIX). The resin can be used, under mild elution conditions, for purifying FIX from plasma as well as from recombinant sources. IXSelect is part of Cytiva's Custom Designed Media program (Fig 1).

Benefits of IXSelect include:

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

FIX is a serine protease that is essential for blood clotting. A deficiency in functional FIX results in hemophilia B, a coagulation disorder characterized by impaired clot formation and prolonged bleeding. The treatment for hemophilia B commonly involves factor replacement by intravenous infusion of recombinant or plasma-derived FIX. IXSelect affinity chromatography resin is specifically designed for the purification of FIX. IXSelect resin enables efficient capture or intermediate purification of FIX, with high purity and yield in large-scale production. Obtained specific FIX activity after purification is approximately 250 IU/mL.

Fig 1. IXSelect chromatography resin is designed for affinity purification of FIX in industrial processes.

Resin characteristics

IXSelect is based on porous, spherical agarose particles (the base matrix) with a covalently attached FIX 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 2). The FIX 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 FIX molecule. The gene of the selected protein was cloned into a yeast cell expression system.

The characteristics of IXSelect are summarized in Table 1.

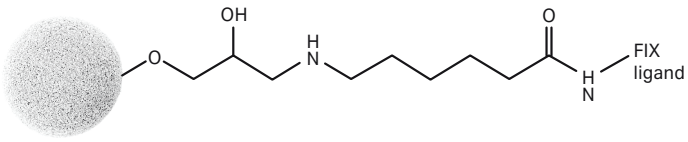


Fig 2. Structure of IXSelect .

Table 1. Main characteristics of IXSelect

Matrix	Highly cross-linked agarose, spherical
Particle size, d_{50v} *	75 µm
Ligand	Recombinant protein (M_r 13 151) produced in *Saccharomyces cerevisiae*
Ligand concentration	Approx. 8 mg/mL resin
Total binding capacity†	Approx. 6 mg FIX/mL resin
Flow velocity	Minimum 600 cm/h in a 1 m diameter 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 20 mM Tris-HCl, 150 mM NaCl, pH 7.4 on HiScale™ 16/20 column. The binding capacity is obtained by measuring the amount of bound and eluted protein in 20 mM Tris-HCl, 2 M $MgCl_2$, pH 7.4.
‡ 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 build-up of contaminants over time, more rigorous protocols may need to be applied (see *Cleaning in place [CIP] and sanitization in place [SIP]*).

Application

Binding and elution conditions

Recommended conditions that will bind and elute FIX with high yield and purity are as follows:

Equilibration, loading, and wash buffer 1: 20 mM Tris-HCl, 150 mM NaCl, pH 7.4

Wash buffer 2: 20 mM Tris-HCl, 500 mM NaCl, 0.01% Tween™ 80, pH 7.4

Elution buffer: 20 mM Tris-HCl, 2 M $MgCl_2$, pH 7.4

Regeneration buffer: 0.1 M glycine, 0.1 M NaCl, pH 2.0

1. Pack the column with IXSelect
2. Equilibrate with equilibration buffer
3. Load the sample
4. Wash with wash buffers 1 and 2
5. Elute with elution buffer
6. Regenerate the column with regeneration buffer
7. Perform CIP

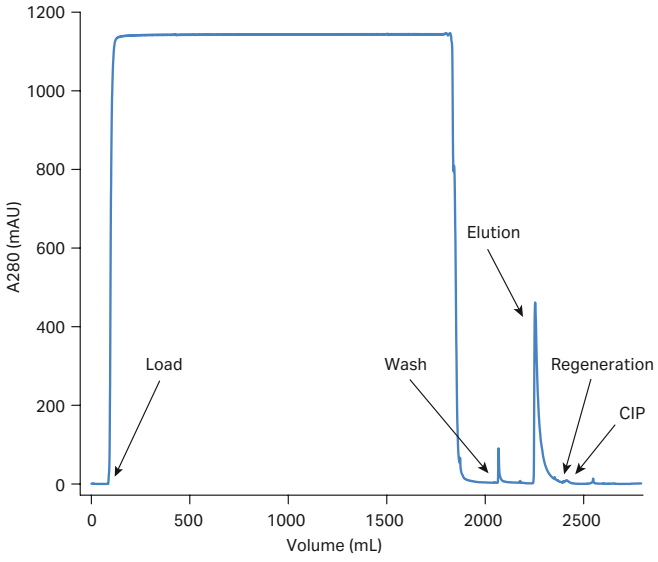
Note! The elution buffer, containing 2 M $MgCl_2$, needs to be exchanged before specific FIX activity measurements as $MgCl_2$ will interfere with the results.

Regeneration restores the function of the resin.

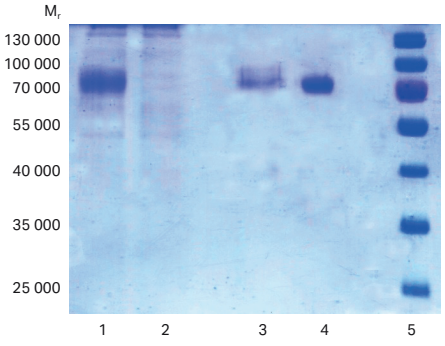
Capture of FIX

Capture of FIX from a Chinese hamster ovary (CHO) cell lysate was performed using IXSelect resin (Fig 3A). Fractions from the FIX capture step were analyzed by SDS-PAGE using a FIX reference preparation as standard (Fig 3B). The identity of the target protein was confirmed by Western blot analysis (Fig 3C).

(A) *Sample:* FIX-containing CHO cell lysate
Column: HiScale 16/20 packed with 13 mL IXSelect resin
Equilibration: 6 column volumes (CV) of 20 mM Tris-HCl, 150 mM NaCl, pH 7.4
Sample load: 1500 mL sample feed loaded at 100 cm/h
Wash: 10 CV of equilibration buffer
10 CV of 20 mM Tris-HCl, 500 mMNaCl, 0.01% Tween 80, pH 7.4
3 CV of equilibration buffer
Elution: 20 mM Tris-HCl, 2 M $MgCl_2$, pH 7.4
Regeneration: 0.1 M glycine buffer
CIP: 10 mM NaOH

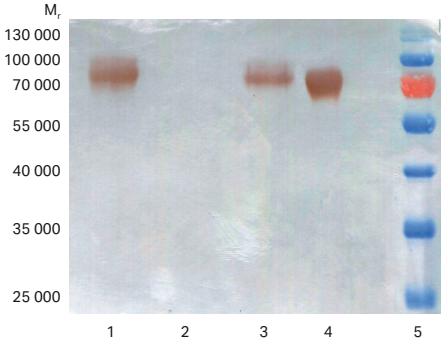


(B)



Lane 1. Cell culture resin
Lane 2. Flowthrough
Lane 3. Elution peak
Lane 4. FIX standard
Lane 5. Low molecular weight marker

(C)



Lane 1. Cell culture resin
Lane 2. Flowthrough
Lane 3. Elution peak
Lane 4. FIX standard
Lane 5. Low molecular weight marker

Fig 3. (A) FIX capture using IXSelect. (B) SDS-PAGE analysis of fractions from FIX capture step. (C) Western blot analysis of fractions from FIX capture step. Data from customer evaluation.

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 IXSelect 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 12.

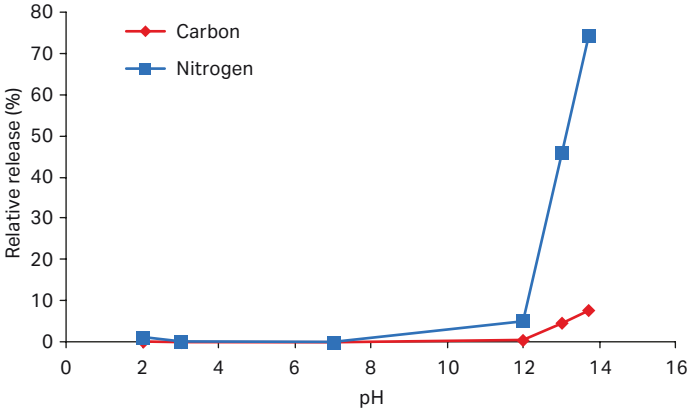


Fig 4. Stability of IXSelect as determined by carbon and nitrogen release after storage at 40˚C for one week in solutions of various pH values.

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 IXSelect 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 IXSelect resin, the Thermo Scientific CaptureSelect IXSelect 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 IXSelect resin in 20% ethanol at 2˚C to 8˚C. The resin is supplied as a suspension in 20% ethanol.

Ordering information

Product	Quantity	Product code
IXSelect	25 mL	17371401
IXSelect	200 mL	17371402
IXSelect	1 L	17371403
IXSelect	5 L	17371404
HiScreen™	1 × 4.7 mL	17371410
HiTrap™ IXSelect	5 × 1 mL	17371411
HiTrap IXSelect	1 × 5 mL	17371412

Related literature	Product code
IXSelect Regulatory Support File	on request
Sofer, G. and Hagel, L. Cleaning, sanitization, and storage, in *Handbook of Process Chromatography: A Guide to Optimization, scale-up and validation*. Academic Press, Amsterdam, pp. 188–214 (1997)	18112156
Handbook: Affinity Chromatography, principles and methods	18102229

cytiva.com/bioprocess

CY13705-13Aug20-DF