GCSFSelect

AFFINITY CHROMATOGRAPHY

Granulocyte-colony stimulating factor (G-CSF) is a hormone that stimulates the bone marrow to an increased production of white blood cells. GCSFSelect affinity chromatography resin is specifically designed for the purification of recombinantly produced G-CSF from various expression systems. GCSFSelect resin enables efficient, initial purification of G-CSF, with high purity and yield at large-scale production. GCSFSelect is part of Cytiva's Custom Designed Media program (Fig 1).

Benefits of GCSFSelect include:

- Efficient, industrial-scale purification of GCSF 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 GCSF for clinical applications

Resin characteristics

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

The characteristics of GCSFSelect are summarized in Table 1.

Fig 1. GCSFSelect chromatography resin is designed for affinity purification of G-CSF in industrial processes.

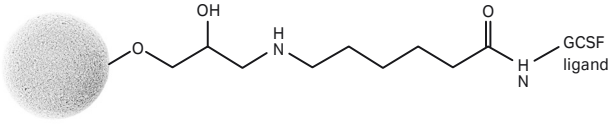


Fig 2. Structure of GCSFSelect.

Table 1. Main characteristics of GCSFSelect

Matrix	Highly cross-linked agarose, spherical
Particle size, d_{50v}*	75 µm
Ligand	Recombinant protein (M_r 14 400) produced in *Saccharomyces cerevisiae*
Ligand concentration	Approx. 7.5 mg/mL resin
Total binding capacity†	Approx. 3.9 mg GCSF/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 PBS, pH 7.4 on Tricorn™ 5/20 column. The binding capacity is obtained by measuring the amount of bound and eluted protein in 20 mM Bis-Tris, 0.08% Tween™ 20, 1 M $MgCl_2$, pH 7.0.

‡ 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 samples. 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])

Application

Screening of running conditions

To mimic a real purification process, recombinant G-CSF was spiked in a PBS-buffered *E. coli* lysate. GCSFSelect packed in a 96-well plate was used to screen for efficient binding and elution conditions. The conditions evaluated are shown in Figure 3.

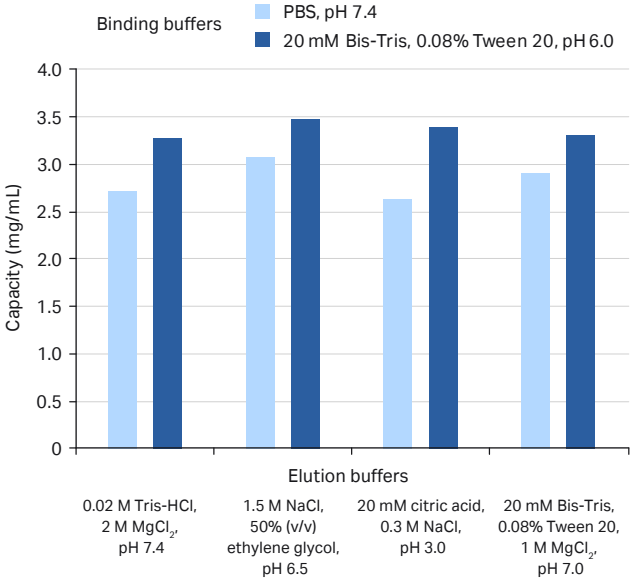
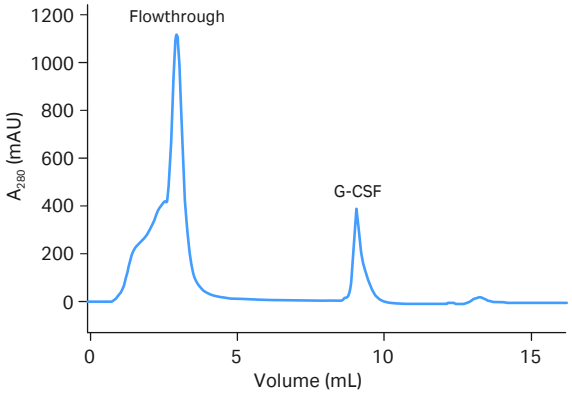


Fig 3. Running conditions evaluated in 96-well plate format.

For highest purity and yield, a PBS buffer (10 mM, pH 7.4) was chosen for the binding step and a Bis-Tris buffer (20 mM, 0.08% Tween, 1 M $MgCl_2$, pH 7.0) for the elution step. The conditions were confirmed in a column-purification study. Figure 4A displays a chromatogram of the running conditions for which the highest medium capacity as well as highest product purity and yield were obtained. Fractions from the purification were further analyzed by SDS-PAGE (Fig 4B).

(A)

Column:	Tricorn 5/20 packed with 0.4 mL GCSFSelect
Sample:	1.65 mL homogenized and clarified *E. coli* lysate spiked with 2.5 mg/mL recombinant G-CSF
Equilibration, loading, and wash buffer:	PBS, pH 7.4
Elution buffer:	20 mM Bis-Tris, 0.08% Tween 20, 1 M $MgCl_2$, pH 7.0
Cleaning-in-place solution:	0.1 M glycine, pH 2.0
System:	ÄKTA™ chromatography system



(B)

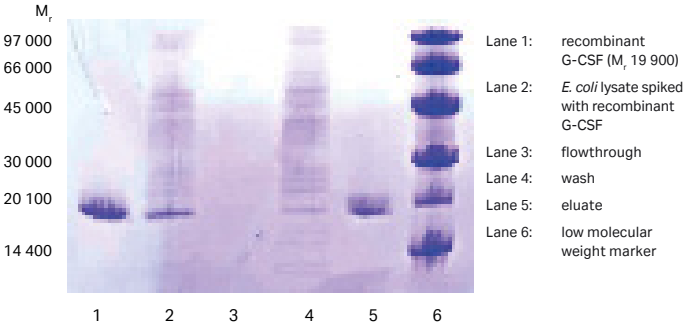


Fig 4. (A) Purification of G-CSF from a G-CSF-spiked *E. coli* lysate. (B) SDS-PAGE of the different fractions from G-CSF affinity purification using GCSFSelect.

Binding and elution conditions

Recommended conditions that will bind and elute G-CSF from a G-CSF-spiked *E. coli* lysate with high yield and purity are as follows:

Equilibration, loading, and wash buffer: PBS, pH 7.4

Elution buffer: 20 mM Bis-Tris, 0.08% Tween 20, 1 M $MgCl_2$, pH 7.0

1. Pack the column with medium.
2. Equilibrate with 10 column volumes (CV) of equilibration buffer.
3. Load the sample.
4. Wash with washing buffer.
5. Elute with 5 to 10 CV of elution buffer.
6. Perform cleaning-in-place.
7. Regeneration.

Stability

For high chemical stability and low ligand leakage, the affinity ligand is immobilized on the agarose base matrix via stable amide bonds. Figure 5 shows the stability of GCSFSelect after storage in different solutions of various pH at 40°C for one week. At pH values > 12, both carbon and nitrogen are released, which indicates hydrolysis of the ligand. Leakage is low in the pH range 2 to 12.

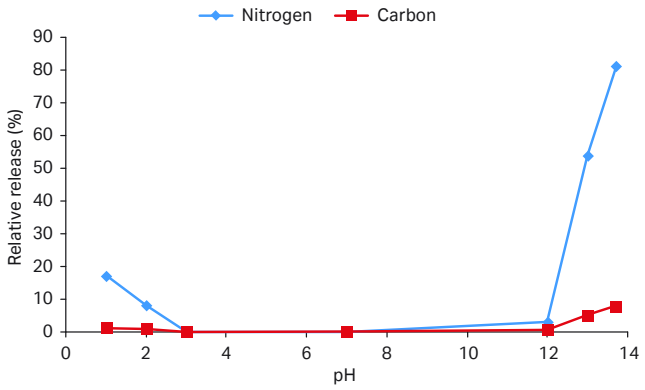


Fig 5. Stability of GCSFSelect after storage at various pH at 40°C for one week as determined by carbon and nitrogen release.

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 GCSFSelect 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 GCSFSelect resin, the Thermo Scientific CaptureSelect GCSFSelect 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 storing GCSFSelect medium in 20% ethanol at 2°C to 8°C. The medium is supplied as a suspension in 20% ethanol.

Ordering information

Product	Pack size	Product code
GCSFSelect	25 mL	17548301
GCSFSelect	200 mL	17548302
GCSFSelect	1 L	17548303
GCSFSelect	5 L	17548304
HiTrap™ GCSFSelect	5 × 1 mL	17548311
HiTrap GCSFSelect	1 × 5 mL	17548312

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Related literature	Product code
GCSFSelect 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

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