IMAC Sepharose 6 Fast Flow resin

Product Information

Cat#No#	IM-369C

Product Overview

IMAC Sepharose 6 Fast Flow is an uncharged IMAC resin for purifying proteins and peptides with affinity for metal ions in batch mode or when scale up is needed:
Supplied uncharged, for customized metal ion charging and optimized selectivity.
Suitable for purification of histidine-tagged (his-tagged) proteins when nickel is not the best choice of metal ion.
Based on well-established Sepharose Fast Flow resins. High chemical stability enables proven CIP and sanitization protocols. Hydrophilic base matrix ensures low levels of non-specific binding and low levels of host cell-derived impurities in the elution pool.

Description

IMAC Sepharose 6 Fast Flow is supplied free of metal ions, allowing the user to charge it with the most appropriate metal ion for purification of a target protein.

Characteristic

Convenient purification of histidine-tagged proteins when Ni^{2+} is not the best choice of metal ion.
Charge with your metal ion of choice to optimize selectivity.
High binding capacity.
BioProcess medium designed to meet manufacturing needs for security-of-supply, robust performance, and regulatory support.
Available in prepacked HiTrap columns for convenient purification and prepacked HiPrep columns for easy scale-up.

Maximum operating pressure

1 bar (14.5 psi, 0.1 MPa) (when packed in XK columns; may vary if used in other columns)

Sample preparation

(Histidine)6 -tagged proteins: Capacity data were obtained for a protein (Mr 28 000) bound from an E. coli extract, and a pure protein (Mr 43 000; applied at 1 mg/ml in binding buffer; capacity at 10% breakthrough).

Tel: 1-631-559-9269 1-516-512-3133
Email:info@creative-biomart.com
Fax:1-631-938-8127
45-1 Ramsey Road, Shirley, NY 11967, USA

CREATIVE BIOMART®
Chromatography

IMAC Sepharose 6 Fast Flow resin

Untagged protein: Capacities determined at 10% breakthrough for human apotransferrin applied at 1 mg/ml in binding buffer.

Metal ion capacity

~25 μmol Cu^{2+}/ml medium, ~15 μmol Ni^{2+} or Zn^{2+}/ml medium

Packing Column

IMAC Sepharose 6 Fast Flow is supplied preswollen in 20% ethanol. Prepare a slurry by decanting the 20% ethanol solution and replacing it with distilled water in a ratio of 75% settled medium to 25% distilled water.

Matrix

6% cross-linked agarose

Particle Size

45 μm-165 μm

Average particle size

~90 μm

Dynamic binding capacity

~40 mg histidine-tagged protein/mL resin when changed with Ni^{2}

Recommended flow rate

150 cm/h

Chemical stability

Stable in commonly used aqueous buffers - 0.01 M HCl, 1 M NaOH, 70% acetic acid, 2% SDS. 30% 2-propanol

pH working range

3–12

CIP stability

3–12

Storage

Tel: 1-631-559-9269 1-516-512-3133

Email:info@creative-biomart.com

Fax:1-631-938-8127

45-1 Ramsey Road, Shirley, NY 11967, USA

IMAC Sepharose 6 Fast Flow resin

4 to 30°C, 20% Ethanol
Binding buffer
20 mM sodium phosphate, 0.5 M NaCl, 5 mM imidazole (1 mM for untagged protein), pH 7.4.
Elution buffer
20 mM sodium phosphate, 0.5 M NaCl, 0.5 M imidazole (50 mM for untagged protein), pH 7.4.
Binding
The choice of binding buffer depends on the metal ion and on the binding properties of the sample molecules. Protein binding to an immobilized metal ion usually occurs in the pH range of 5.5 to 8.5. Binding is often strongest at the upper end of this range. For histidine-tagged protein applications, imidazole at low concentrations is commonly used in the samples as well as in the binding/wash buffer to minimize binding of unwanted host cell proteins. The concentration of imidazole that will give optimal purification results is protein-dependent. For IMAC Sepharose 6 Fast Flow charged with Ni2+ or Co2+, 20 to 40 mM in the sample as well as in the binding and wash buffer is a good starting point for optimization.
Elution
Elution is performed by reducing the pH or by competitive displacement, using for example imidazole. The most frequently used elution procedure for histidine-tagged proteins is based on a linear or stepwise increase of the imidazole concentration. Elution by reducing pH can be performed using a linear or stepwise gradient. Most untagged proteins can be eluted between pH 6 and 4. Prepacked HiTrap IMAC FF columns are an excellent choice for screening to establish the optimal chromatographic conditions.
Regeneration
When performing repeated purification cycles, the need for stripping and re-charging is dependent on the sample properties, sample volumes, metal ion, etc.
Cleaning-in-place
In some applications, substances such as denatured proteins or lipids are not removed during the regeneration procedures. These substances can be removed by Cleaning-In-Place (CIP). The column should

Tel: 1-631-559-9269 1-516-512-3133
Email:info@creative-biomart.com
Fax:1-631-938-8127
45-1 Ramsey Road, Shirley, NY 11967, USA

IMAC Sepharose 6 Fast Flow resin

be cleaned when the back pressure increases, or to avoid cross-contamination between samples/target proteins.

Purification procedures
1.Charge the packed column with metal ions according to the procedure described earlier. 2.If the column has been stored in 20% ethanol after metal ion charging, wash it with 2–5 column volumes (CV) of distilled water. Use a linear flow rate of 50–100 cm/h. 3.Equilibrate the column with at least 2–5 CV of binding buffer at a linear flow rate of 150 cm/h or higher. 4.Apply a correct prepared sample. 5.Wash out unbound material with binding buffer until the absorbance is at or near the baseline. 6.Elute the bound protein with elution buffer using a stepwise or linear gradient.
Pack size
25 mL
BioProcess resin
Yes
Maximum flow velocity
600 cm/h (20 mL/min) using XK 16/20 columns with 5 cm bed height
Column volume
0.25 ml or 1 ml.
Column hardware pressure limit
5 bar (0.5 MPa, 72 psi)

Tel: 1-631-559-9269 1-516-512-3133

Email:info@creative-biomart.com

Fax:1-631-938-8127

45-1 Ramsey Road, Shirley, NY 11967, USA