CREATIVE BIOMART®
Chromatography

HiScreen IMAC FF

Product Information

Cat#No#	Hi-066P

Product Overview

HiScreen IMAC FF are prepacked with IMAC Sepharose 6 Fast Flow, a resin which is charged with the metal of choice for purification of histidine-tagged proteins. The HiScreen format is optimized for method optimization and parameter screening.

Description

IMAC Sepharose 6 Fast Flow consists of 90 µm beads of highly cross-linked agarose to which a chelating group has been covalently coupled. This chelating group can be charged with suitable metal ions, specific for your target protein of interest.

Characteristic

For optimizing purification of histidine-tagged proteins when Ni2+ is not the best choice of metal ion.
Conveniently charge with your metal ion of choice.
Optimal bead size for scale-up.
High binding capacity.
Prepacked HiScreen columns for method optimization and process development.
Reproducible results, scalable to BioProcess columns packed with the same resin using the same linear fluid velocity.

Maximum operating pressure

1.5 bar [0.15 MPa] (22 psi)

Metal ion capacity

Approx. 15 µmol Ni2+ /ml medium

Matrix

Highly cross-linked 6% agarose

Average particle size

90 µm

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Dynamic binding capacity
Approx. 40 mg (histidine)6-tagged protein/ml medium (Ni2+-charged). Untagged protein: Approx. 25 mg/ml medium (Cu2+ charged), or approx. 15 mg/ml medium (Zn2+ or Ni2+ charged).
Recommended flow rate
30 to 300 cm/h
Recommended column height
100 mm
Chemical stability
1 M NaOH, 70% acetic acid. Tested for 12 h. 2% SDS. Tested for 1 h. 30% 2-propanol. Tested for 30 min.
pH working range
3 to 12
CIP stability
2 to 14
Storage
4 to 30°C, 20% Ethanol
Binding buffer
20 mM sodium phosphate, 500 mM NaCl, 5 mM imidazole, (1 mM imidazole for untagged protein), pH 7.4.
Elution buffer
20 mM sodium phosphate, 0.5 M NaCl, 500 mM imidazole, pH 7.4.
Cleaning-in-place
Ionically bound proteins: 1. Wash with several column volumes (CV) of 1.5 M NaCl. 2. Wash with at least 3 CV distilled water. Precipitated proteins, hydrophobically bound proteins, and lipoproteins: 1. Wash the column with 1 M NaOH, contact time usually 1 to 2 h (longer time may be required to inactivate endotoxins). 2. Wash with approximately 3 to 10 CV start buffer. 3. Wash with 5 to 10 CV distilled water.

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Hydrophobically bound proteins, lipoproteins, and lipids: 1. Wash with 5 to 10 CV 30% isopropanol for at least 15 to 20 min. 2. Wash with approximately 10 CV distilled water. or 1. Wash with 2 CV detergent in a basic or acidic solution. Use, for example, 0.1 to 0.5% nonionic detergent in 0.1 M acetic acid, contact time 1 to 2 h. 2. Remove residual detergent by washing with at least 5 CV 70% ethanol. 3. Wash with 3 to 10 CV start buffer.

Scaling up

After optimizing the method at laboratory scale, the process is ready for scaling up.
For quick scale-up of purification, two HiScreen columns can easily be connected in series with a union to give 20 cm bed height. Note that the back pressure will increase. This can easily be addressed by lowering the flow rate.
Factors, such as the clearance of critical impurities, may change when column bed height is modified and should be validated using the final bed height.
Scale-up is typically performed by keeping bed height and flow velocity (cm/h) constant while increasing bed diameter and flow rate (mL/min or L/h).

Purification procedures

1. After the column preparation (charging with metal ions), equilibrate with at least 5 column volumes (CV) of start buffer. Avoid introducing air into the column.
2. Adjust the sample to the chosen starting conditions and load on the column.
3. Wash with 5 to 10 CV start buffer until the UV trace of the effluent returns to near baseline.
4. Elute either by linear gradient elution or a step elution at recommended flow rates.
5. If required, strip the column from metal ions and perform a CIP to clean the column.
6. Re-equilibrate the column with 5 to 10 CV start buffer or until the UV baseline, eluent pH, and conductivity reach the required values.

Pack size

4.7 mL

Maximum flow velocity

600 cm/h

Dimensions

7.7 × 100 mm

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Column volume
4.7 ml
Column i.d.
7.7 mm
Column hardware pressure limit
0.8 MPa (8 bar, 116 psi)

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