HiTrap IgM Purification HP

Product Information

Cat#No#	Hi-012P

Product Overview

HiTrap IgM Purification HP is a 1 mL column prepacked with Sepharose High Performance for fast and efficient purification of monoclonal IgM.

Description

HiTrap IgM Purification HP is prepacked with a thiophilic adsorption resin, 2-mercaptopyridine coupled to Sepharose High Performance. Thiophilic adsorption is promoted by water-structuring salts. It has been suggested that the interaction of IgM and ligand results from combined electron donating and accepting action of the ligand or, alternatively, a mixed mode hydrophilic-hydrophobic interaction.

Characteristic

Optimized for fast, efficient purification of monoclonal IgM from hybridoma cell culture.
Prepacked with a thiophilic adsorption resin, 2-mercaptopyridine coupled to Sepharose High Performance.
Recovery of IgM is high with excellent activity retention.
The top and bottom frits are manufactured from porous polyethylene.
Columns are delivered with a stopper on the inlet and a snap-off end on the outle.

Applications

Used for purification of IgM.

Maximum operating pressure

5 bar [0.5 MPa] (70 psi)

Sample preparation

The sample must have the same concentration of ammonium sulphate as the binding buffer. Perform buffer exchange using HiTrap Desalting, HiPrep 26/10 Desalting, or PD-10 Desalting columns or gradually add small amounts of solid ammonium sulphate to the sample until the final concentration is, for example, 0.8 M. Stir slowly and continuously during this procedure. Pass the sample through a 0.45 µm filter immediately before loading on the column.

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CREATIVE BIOMART®
Chromatography

HiTrap IgM Purification HP

Matrix
Highly cross-linked spherical agarose
Average particle size
34 µm
Ligand
2-mercaptopyridine
Ligand density
2 mg/ml medium
Dynamic binding capacity
5 mg human IgM/ml medium
Recommended flow rate
0.1–1 ml/min
Recommended column height
25 mm
pH working range
3 to 11
CIP stability
2 to 13
Temperature stability
4°C to room temperature
Storage
4°C to 8°C.
Shipping
20% ethanol

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Binding buffer
20 mM sodium phosphate, 0.8 M (NH4)2SO4, pH 7.5.
Elution buffer
20 mM sodium phosphate, pH 7.5.
Binding
Not all monoclonal IgM may bind to the HiTrap IgM Purification HP column at 0.8 M ammonium sulphate. Binding can be improved by increasing the ammonium sulphate concentration to 1.0 M. However, an increased concentration of ammonium sulphate will cause more IgG to bind, which might be a problem if the sample is serum or if serum has been added to the cell culture medium. If the purified IgM is contaminated by IgG, the IgG can be removed by using for example HiTrap rProtein A FF. Alternatively, the ammonium sulphate can be exchanged for 0.5 M potassium sulphate. Most monoclonal IgM bind to the column in the presence of 0.5 M potassium sulphate and the purity of IgM is comparable to the purity achieved with 0.8 M ammonium sulphate.
Elution
Some monoclonal IgM may bind too tightly to HiTrap IgM Purification HP for total elution with elution buffer. The remaining IgM will be eluted with the regeneration buffer, but the high content of isopropanol will cause precipitation of IgM and immediate buffer exchange, using HiTrap Desalting, PD-10 Desalting column or dilution of the sample is required to preserve the IgM. Lower concentrations of isopropanol may elute the IgM and decrease the risk of precipitation.
Regeneration
20 mM sodium phosphate, pH 7.5 with 30% isopropanol.
Purification procedures
1. Fill the syringe or pump tubing with buffer. Remove the stopper and connect the column to the syringe (with the provided luer connector), or pump tubing, ‘‘drop to drop” to avoid introducing air into the column. 2. Remove the snap-off end at the column outlet. 3. Wash the column with 5 column volumes of each buffer: Binding buffer, elution buffer and regeneration buffer.

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4. Equilibrate the column with 5 column volumes of binding buffer.
5. Apply the sample using a syringe fitted to the luer connector, or by pumping it onto the column.
6. Wash out unbound sample with 15 column volumes of binding buffer or until no material appears in the effluent.
7. Elute the IgM with 12 column volumes of elution buffer.
8. Regenerate the column with 7 column volumes of regeneration buffer.
9. Re-equilibrate the column with 5 column volumes of binding buffer.

Pack size
5 × 1 mL
Dimensions
7 × 25 mm
Column volume
1 ml
Column i.d.
7 mm
Column hardware pressure limit
5 bar (0.5 MPa, 70 psi)

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