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A Geno Technology, Inc. (USA) brand name

G-Sep™ Ion Exchange Agarose Fast Flow

CM, DEAE, Q & SP Agarose Fast Flow

(Cat. # 786-965, 786-966, 786-967, 786-968, 786-969, 786-970, 786-971, 786-972)

INTRODUCTION

G-Sep™ Ion Exchange Agarose Fast Flow (FF) resins are available with the weak exchange groups DEAE and CM, and the strong exchange groups Q and SP attached to a highly cross-linked 6% agarose beads. The resins are extensively used for biomacromolecule separations in both research and industrial applications. The G-Sep™ Ion Exchange Agarose Fast Flow (FF) resins have high chemical stability, allowing well proven cleaning-in-place (CIP) and sanitization protocols and have been the industrial standard for ion exchange chromatography during recent decades.

G-Sep™ CM Agarose Fast Flow is a weak cation exchanger composed of highly cross-linked 6% agarose beads, with Carboxymethyl (CM) weak cation exchange groups.

G-Sep™ DEAE Agarose Fast Flow is a weak anion exchanger composed of highly cross-linked 6% agarose beads, with diethylaminoethyl (DEAE) weak anion exchange groups.

G-Sep™ Q Agarose Fast Flow is a strong anion exchanger composed of highly cross-linked 6% agarose beads, with quaternary ammonium (Q) strong anion exchange groups.

G-Sep™ SP Agarose Fast Flow is a strong cation exchanger composed of highly cross-linked 6% agarose beads, with Sulphopropyl (SP) strong cation exchange groups.

ITEM(S) SUPPLIED

Cat. #	Description	Size
786-965	G-Sep™ CM Agarose Fast Flow	25ml
786-966	G-Sep™ CM Agarose Fast Flow	500ml
786-967	G-Sep™ DEAE Agarose Fast Flow	25ml
786-968	G-Sep™ DEAE Agarose Fast Flow	500ml
786-969	G-Sep™ Q Agarose Fast Flow	25ml
786-970	G-Sep™ Q Agarose Fast Flow	300ml
786-971	G-Sep™ SP Agarose Fast Flow	25ml
786-972	G-Sep™ SP Agarose Fast Flow	300ml

STORAGE CONDITIONS

It is shipped at ambient temperature. Upon arrival, store at 4°C. DO NOT FREEZE.

SPECIFICATIONS

Cation Exchangers

	G-Sep™ CM Agarose	**G-Sep™ SP Agarose**
Matrix	Cross-linked agarose beads, 6%	
Ligand	Carboxymethyl	Sulphopropyl
Ion Exchanger	Weak cation exchanger	Strong cation exchanger
Bead form	Spherical, diameter 50-160µm	Spherical, diameter 45-165µm
Ionic Capacity	0.09-0.13 mmol (H^+)/ml	0.18-0.25 mmol (Na^+)/ml
Binding Capacity	70 mg lysozyme /ml medium	
pH stability Working Range	4-12	2-12
pH stability Cleaning-in-Place (CIP)	2-14	
Maximum Flow Velocity	450cm/h	
Maximum Pressure	0.3MPa	
Exclusion limit(globular proteins)	4×10^6	
Physical Stability	Negligible volume variation due to changes in pH or ionic strength	
Chemical Stability	Stable to all commonly used aqueous buffers:1 M NaOH, 8M urea, 8M guanidine hydrochloride, 70% ethanol	
Autoclavable	With Na^+ as counter Ions, at 121 °C, pH 7, for 30 min in 0.2M sodium acetate for autoclaving	
Storage Conditions	4 to 30°C, 20% Ethanol	4 to 30°C, 20% Ethanol containing 0.2M sodium acetate

Anion Exchangers

	G-Sep™ DEAE Agarose	G-Sep™ Q Agarose
Matrix	Cross-linked agarose beads, 6%	
Ligand	Diethylaminoethyl	Quaternary ammonium
Ion Exchanger	Weak anion exchanger	Strong anion exchanger
Bead form	Spherical, diameter 50-160µm	
Ionic Capacity	0.11-0.16mmol (Cl^-)/ml	0.18-0.25mmol (Cl^-)/ml
Binding Capacity	90mg HSA/ml medium	120mg HSA/ml medium
pH stability Working Range	2-9	2-12
pH stability Cleaning-in-Place (CIP)	2-14	
Maximum Flow Velocity	450cm/h	
Maximum Pressure	0.3MPa	
Exclusion limit(globular proteins)	4×10^6	
Physical Stability	Negligible volume variation due to changes in pH or ionic strength	
Chemical Stability	Stable to all commonly used aqueous buffers:1M NaOH, 8M urea, 8M guanidine hydrochloride, 70% ethanol	
Autoclavable	With Cl^- as counter Ions, at 121°C, pH 7, for 30 min	
Storage Conditions	4 to 30°C, 20% Ethanol	

PREPARING THE MEDIUM

G-Sep™ Ion Exchange Agarose Fast Flow (FF) resins are supplied in a solution containing 20% ethanol.

Prepare a 75% slurry using a binding/ eluent buffer of choice. The slurry will be 75% settled resin and 25% buffer. It is recommended to degas the slurry before packing.

PACKING G-SEP™ AGAROSE FAST FLOW

1. Equilibrate all material to room temperature.
2. De-gas the slurry
3. Eliminate air from the column dead spaces by flushing the end pieces with buffer. Make sure no air has been trapped under the column net. Close the column outlet with a few centimeters of buffer remaining in the column.
4. Pour the gel slurry into the column in one continuous motion. Pouring the slurry down a glass rod held against the wall of the column will minimize the introduction of air bubbles.
5. Fill the remainder of the column with buffer, mount the column top piece onto the column and connect the column to a pump.
6. Open the bottom outlet of the column and set the pump to run at the desired flow rate. This should be at least 133% of the flow rate to be used during subsequent chromatographic procedures. However, the maximum flow rate is typically employed during packing.
7. Maintain the packing flow rate for 3 bed volumes after a constant bed height is reached.

Using an adapter

1. After the medium have been packed as described above, close the column outlet and remove the top piece from the column. Carefully fill the rest of the column with buffer to form an upward meniscus at the top.
2. Insert the adaptor into the top of the column at an angle, taking care not to trap air under the net.
3. Make all tubing connections at this stage. There must be a bubble-free liquid connection between the column and the pump.
4. Slide the plunger slowly down the column so that the air above the net and in the capillary tubings is displaced by eluent. Valves on the inlet side of the column should be turned in all directions during this procedure to ensure that air is removed.
5. Lock the adapter in position on the medium surface, open the column outlet and start the eluent flow. Pass eluent through the column at the packing flow rate until the bed is stable. Re-position the adapter on the medium surface as necessary.
6. The column is now packed and equilibrated and ready for use.

OPERATION

Equilibration

Equilibrate the column with the starting buffer when the pH and/or conductivity of the effluent is the same as the starting buffer.

Sample preparation

The amount of sample that can be applied to the column depends on the available capacity of the ion exchanger and the degree of resolution required. For best resolution it is usually not advisable to exceed 10 to 20% of the available capacity.

The sample should be dissolved in start buffer. The viscosity of the sample should not exceed that of the buffer. For normal aqueous buffer systems, this corresponds to a protein concentration of approximately 50 mg/ml. Before application the sample should be centrifuged or filtered through a 0.45µm filter to remove any particulate matter.

Operating flow rates

The typical linear flow rate of G-Sep™ Ion Exchange Agarose Fast Flow (FF) resins is 300-400cm/h through 15 cm bed height at a pressure of 0.1MPa.

Binding

For efficient binding, the ionic strength of the starting buffer must be low. The pH should be at least one pH unit different from the isoelectric point (pI) of the molecules to be bound and within 0.5 pH units of the selected buffer salt's pKa.

For G-Sep™ CM Agarose and G-Sep™ SP Agarose, the starting buffer must be at least one pH unit ***below*** the pI of the molecule to be bound.

For G-Sep™ DEAE Agarose and G-Sep™ Q Agarose, the starting buffer must be at least one pH unit ***above*** the pI of the molecule to be bound.

Elution

A linear gradient of increasing sodium chloride concentration is the most commonly used elution method in ion exchange. A suggested gradient is from start buffer containing no NaCl to start buffer containing 0.5M NaCl. Increase the salt concentration if the substance of interest is not eluted in the gradient.

Regeneration

After every run, very tightly bound material is eluted using either high ionic (e.g.1M NaCl) strength or a change in pH. The medium is re-equilibrated with starting buffer before each run.

Cleaning-in-place(CIP)

Remove ionically bound proteins: Wash with filtered 2M NaCl in a reversed flow direction. Contact time 10-15min.

Remove precipitated proteins and hydrophobically bound proteins or lipoproteins: Wash with 1M NaOH. Contact time 1-2 hours.

Lipids and very hydrophobic proteins: Wash with 70% ethanol, reversed flow for 1-2 hours. Alternatively wash with saw-tooth gradients of 0-30% isopropanol. Contact time 1-2 hours.

After cleaning, equilibrate the column with sterile start buffer before use.

Sanitization

Wash the column in the reversed flow direction with 0.5-1M NaOH for 30-60 min. Re-equilibrate the column with sterile start buffer. Sanitization is the use of chemical agents to inactivate microbial contaminants in the form of vegetative cells; it also helps to maintain a high level of both process hygiene and process economy.

Storage

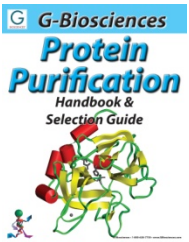
G-Sep™ Ion Exchange Agarose Fast Flow resins should be stored in the salt form in a buffer containing 20% ethanol. Recommended storage at 4 to 30°C. Do not freeze.

Shelf life

5 year

RELATED PRODUCTS

Download our Sample Preparation and Protein Purification Handbooks.



http://info2.gbiosciences.com/complete-protein-purification-handbook

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