G-Biosciences ♦ 1-800-628-7730 ♦ 1-314-991-6034 ♦ technical@GBiosciences.com

A Geno Technology, Inc. (USA) brand name

G-Sep™ Butyl Agarose Fast Flow

For Hydrophobic Interaction Chromatography (HIC)

(Cat. # 786-957, 786-958)

INTRODUCTION

G-Sep™ Butyl Agarose Fast Flow (FF) is a 6% cross-linked agarose with butyl groups for Hydrophobic Interaction Chromatography (HIC). The resin is used to separate biomolecules on the basis of relative hydrophobicity.

G-Sep™ Agaroses for HIC are available with butyl, octyl and phenyl groups immobilized on 6% cross-linked agarose beads through neutral and highly stable ether linkages.

ITEM(S) SUPPLIED

Cat. #	Description	Size
786-957	G-Sep™ Butyl Agarose Fast Flow	25ml
786-958	G-Sep™ Butyl Agarose Fast Flow	200ml

STORAGE CONDITIONS

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

SPECIFICATIONS

	Butyl Agarose Fast Flow
Matrix	Highly cross-linked Agarose, 6%
Bead form	Spherical, diameter 50-160µm
Ligand	Butyl
Ligand Concentration	About 40µmol /ml
Binding Capacity	About 20mg HAS/ml resin
pH stability Working Range	3-13
pH stability Cleaning-in-Place (CIP)	2-14
Maximum Pressure (MPa)	0.3
Maximum Flow Velocity	450cm/h
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, 8 M urea, 8 M guanidine hydrochloride, 70% ethanol
Sterilization	Autoclavable, In 1M NaOH,121 °C, pH 7, for 30 min
Storage Conditions	4 to 30°C, 20% Ethanol

PREPARING THE MEDIUM

G-Sep™ Butyl Agarose Fast Flow is 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

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 the Butyl Agarose Beads Fast Flow is 300-400 cm/h through 15 cm bed height at a pressure of 0.1MPa.

Binding

The binding of proteins to hydrophobic media is influenced by:

- Structure of the ligand e.g. carbon chain or an aromatic ligand
- Ligand density
- Ionic strength of the buffer
- Salting-out effect (see The Hofmeister series in Table 2)
- Temperature

Elution

Bound proteins are eluted by reducing the hydrophobic interaction. This can be done by:

- Reducing the concentration of salting out ions in the buffer with a decreasing salt gradient (linear or step)
- Increasing the concentration of chaotropic ions in the buffer in a positive gradient (linear or step)
- Eluting with a polarity-reducing organic solvent, for example ethylene glycol including detergent in the eluent

Regeneration

After every run, elute reversibly bound material with low ionic strength buffer, and wash with H_2O and starting buffer.

Cleaning-in-place(CIP)

Remove precipitated proteins and hydrophobically bound proteins or lipoproteins: Wash with 1M NaOH and immediately rinse with H_2O.

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. Alternatively, wash the column with detergent in a basic or acidic solution. Use e.g. 0.5% non-ionic detergent in 1M acetic acid. Remove residual detergent by washing with 70% ethanol.

Sanitization

Wash the column with 0.5-1M NaOH for 30-60 min. 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

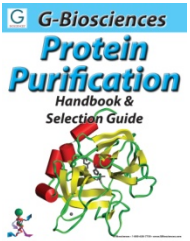
G-Sep™ Butyl Agarose Fast Flow should be stored in the salt form in a buffer containing 20% ethanol. Recommended storage at 4 to 30°C. Do not freeze.

Shelf life

5 year

RELATED PRODUCTS

Download our Sample Preparation and Protein Purification Handbooks.



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

For other related products, visit our website at www.GBiosciences.com or contact us.

Last saved: 4/16/2015 CMH

This page is intentionally left blank

www.GBiosciences.com