G-Trap™ HIC Columns

(Cat. # 786-1001, 786-1002, 786-1003, 786-1004, 786-1005, 786-1006, 786-1007, 786-1008)

INTRODUCTION

G-Trap™ HIC Columns are prepacked ready to use columns for separation of biomolecules via Hydrophobic Interaction chromatography (HIC). Biomolecules are separated on the HIC column based upon the hydrophobicity of the ligand attached to the resin, the distribution of surface-exposed hydrophobic amino acids present on the protein or enzyme to be separated and the concentration and type of salt used in binding or start buffer. In HIC, the binding of biomolecules to the column (ligand) is facilitated by using higher concentrations of anti-chaotropic salts such as ammonium sulfate, sodium sulphate, etc. in the binding buffer.

G-Biosciences offer a wide range of G-Trap™ HIC Columns such as G-Trap™ Butyl Agarose 6 Fast Flow, G-Trap™ Octyl Agarose 6 Fast Flow, G-Trap™ Phenyl Agarose 6 Fast Flow (High Sub) and G-Trap™ Phenyl Agarose 6 Fast Flow (Low Sub) so that one can choose the HIC column that is suitable for separation of the desired protein. Straight alkyl chains ligands such as butyl, octyl show pure hydrophobic character whereas aryl ligands such as phenyl show mixed mode behavior where aromatic, hydrophobic as well as lack of charge play role in protein/biomolecules adsorption to the column. G-Trap™ HIC Columns are fast flow resin columns made from highly cross-linked 6% agarose with mean particle size of 90 µm, covalently coupled to hydrophobic ligand. Some HIC columns such as G-Trap™ Phenyl Agarose 6 Fast Flow (Low Sub) has low ligand substitution to offer a broader range of resins that covers separation of wide range of proteins. The characteristics of the G-Trap™ HIC Columns resins are listed in Table 2

The G-Trap™ columns are made of biocompatible polypropylene, which does not interact with biomolecules. The column has a stopper at the inlet and snap-off end at the outlet. The characteristics of the column are listed in Table1

ITEM(S) SUPPLIED

Cat. #	Description	Size
786-1001	G-Trap™ Butyl Agarose 6 Fast Flow, 1 ml	5 columns
786-1002	G-Trap™ Butyl Agarose 6 Fast Flow, 5 ml	5 columns
786-1003	G-Trap™ Octyl Agarose 6 Fast Flow, 1 ml	5 columns
786-1004	G-Trap™ Octyl Agarose 6 Fast Flow, 5 ml	5 columns
786-1005	G-Trap™ Phenyl Agarose 6 Fast Flow (High Sub), 1 ml	5 columns
786-1006	G-Trap™ Phenyl Agarose 6 Fast Flow (High Sub), 5 ml	5 columns
786-1007	G-Trap™ Phenyl Agarose 6 Fast Flow (Low Sub), 1 ml	5 columns
786-1008	G-Trap™ Phenyl Agarose 6 Fast Flow (Low Sub), 5 ml	5 columns

Connector supplied with the G-Trap™ HIC Column:

Stop plug female, 1/16": This connector is for sealing bottom of G-Trap™ HIC Column. One stop plug female is supplied per column.

STORAGE CONDITIONS

G-Trap™ HIC Columns are shipped at ambient temperature. Upon arrival, store it refrigerated at 4°C, DO NOT FREEZE. This product is stable for 1 year at 4°C. The resin in the column should be stored in 20% ethanol at 4°C after use.

SPECIFICATIONS

Table 1: G-Trap™ HIC Columns

Features	1 ml column	5 ml column
Column Volume	1 ml	5 ml
Column Dimensions	0.7 x 2.5 cm	1.6 x 2.5 cm
Column Hardware Pressure Limit	0.5 MPa	0.5 MPa
Column hardware	Polypropylene	Polypropylene

NOTE: *The pressure over the packed volume varies depending upon the type of medium or matrix, sample or liquid viscosity, and the column tubing used.*

Table 2: G-Trap™ HIC Resin

	G-Trap™ Butyl Agarose 6 Fast Flow	G-Trap™ Octyl Agarose 6 Fast Flow	G-Trap™ Phenyl Agarose 6 Fast Flow (High Sub)	G-Trap™ Phenyl Agarose 6 Fast Flow (Low Sub)
Mean particle size	90 µm			
Bead structure	Highly cross-linked 6% Agarose			
Ligand	Butyl	Octyl	Phenyl	
Ligand Concentration	~40µmol/ml	~5µmol/ml	~40µmol/ml	~25µmol/ml
Binding Capacity	~20mg HSA/ml resin	~30mg HSA/ml resin	~40mg HSA/ml resin	~20mg HSA/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
Storage Conditions	4 to 30°C, 20% Ethanol

IMPORTANT INFORMATION

1. It is essential to do screening of various G-Trap™ HIC Columns to chose the appropriate column. For screening, G-Trap™ HIC Selection Kit (Cat. # 786-1009) is available that offers 4 different columns.
   NOTE: *For most of the proteins hyrophobicity indices are not available. Even if it is available, there are other factors that affect the binding of protein to HIC adsorbant. Therefore it is recommended to screen for the appropriate column for protein separation when doing separation for the first time.*
2. The buffers, salts, and water used during HIC should be of high purity.
3. The G-Trap™ HIC Columns can be operated with syringe, peristaltic pump or a chromatography system.
4. G-Trap™ HIC Columns cannot be opened or refilled.

ADDITIONAL ITEMS REQUIRED

- Union 1/16" male/luer female: For connecting a syringe to G-Trap™ HIC Column.
- G-Trap™ HIC Selection Kit (Cat. # 786-1009) for initial screening
- Start/Binding buffer such as 50 mM sodium phosphate, 1.5 ammonium suphate, pH7
  NOTE: *One can use any binding buffer with any salt depending upon what is best for you (check Table 2)*
- Elution buffer: Eg: 50 mM sodium phosphate, pH7
- 20% ethanol
- Operation unit: syringe or peristaltic pump or a liquid chromatography system
- G-Trap™ GT-600 Desalting Columns (Cat. # 786-1023) or SpinOUT™ GT-600 (Cat. # 786-170) for buffer exchange during sample preparation or buffer exchange of the eluted protein

PROTOCOL

Sample Preparation

1. The sample buffer composition should be adjusted to the start buffer (high salt buffer) composition. If possible dissolve the sample in start buffer. Buffer exchange can be done with start buffer using G-Trap™ GT-600 Desalting Columns (Cat. # 786-1023) or SpinOUT™ GTs (Cat. # 786-170).
   NOTE: *Do not use > 1.5M concentrations of salt as it can shrink the resin)*

2. Remove the particulates from sample either by centrifugation or filter through 0.45 µm filter before loading the sample.
3. High sample viscosity causes high pack pressure. Recommended maximum sample viscosity is equivalent to around 50 mg/ml protein in aqueous solution.

Column equilibration

1. Fill the syringe or pump tubing with elution buffer and remove the stopper. Carefully connect the column "drop to drop" to the syringe (or tubing) via the leur connector to avoid adding air into the column
2. Remove the snap-off end and wash the column with 5 column volumes (CV) of elution buffer at 1 ml/min.
3. Wash the column with 5-10 CV of start buffer. If somehow air bubble is trapped in column wash until its removed.

Purification

1. Equilibrate the column a described above (column equilibration).
2. Apply the sample (Check Sample preparation)
3. Wash with 5-10 CV start buffer till the effluent fraction shows no sign of protein (monitor by UV absorption at 280 nm)
4. Start the gradient elution (10 to 20 CV gradient volume is sufficient)
   NOTE: *A linear decrease of salt concentration is the most commonly used elution method for HIC. Other elution methods can also be used such as increasing the concentration of chaotropic ions in the buffer (linear gradient or step) or eluting with a polarity- reducing organic solvent such as ethylene glycol or including detergent in eluent.*
   NOTE: *In stepwise elution sequential use of same buffer with different ionic strength is applied to the column. It is usually recommended to first characterize the sample with continuous gradient before switching to stepwise elution to avoid any artifactual peaks*
5. Regenerate the column by washing it with 5 CV of distilled water, followed by 5CV start buffer. The column is ready to be used with new.
   NOTE: *The column and the chromatography system should not be stored in high salt buffer to prevent crystal buildup*
   NOTE: *Increased pressure generated when running buffers or samples pass through the resin may affect the packed bed and column hardware and should be avoided. Increased pressure is generated when one or more of the combinations such as high flow rate, high viscosity of buffers or samples, low temperature and flow restrictor are enforced on the column.*

Cleaning and regeneration

G-Trap™ HIC Columns are regenerated by washing with distilled water. However to prevent built-up of contaminants on the column, regular cleaning is advised. Precipitated proteins are removed with 5CV of 1 M NaOH followed by washing with 5-10 CV of water. Strongly bound proteins or lipids are removed by washing the column with 5 to 10 CV of 0-70 % ethanol gradient or 0-30% isopropanol.

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

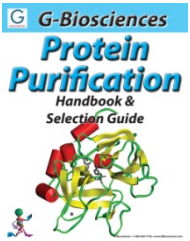
NOTE: *Sanitization of the column can be done with 0.5-1M NaOH for 30-60 minutes to inactivate microbial contaminants.*

STORAGE

G-Trap™ HIC Columns are equilibrated with 5 to 10 CV 20% ethanol before storage. The recommended storage temperature range is 4°C.

RELATED PRODUCTS

Download our Protein Purification Handbook.



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

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