G-Trap™ Ion Exchange Selection Kit

CM, DEAE, Q and SP Agarose Fast Flow columns

(Cat. # 786-1018)

INTRODUCTION

G-Trap™ Ion Exchange Selection Kit contains 4 different types of 1 ml G-Trap™ Ion Exchange Agarose Fast Flow Columns which are used for initial screening of the suitable ion exchange resin and/or pH or ionic strength optimization of start and elution buffer to separate the target protein or substance via ion exchange chromatography. Biomolecules or proteins that carry net charge opposite to that of ion-exchanger bind to that ion exchanger and are thus separated from bulk molecules in the sample. G-Trap™ Ion Exchange Selection Kit contains one of each, G-Trap™ CM Agarose Fast Flow, G-Trap™ SP Agarose Fast Flow, G-Trap™ DEAE Agarose Fast Flow and G-Trap™ Q Agarose Fast Flow column.

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.

G-Trap™ CM Agarose Fast Flow and G-Trap™ SP Agarose Fast Flow contains cation exchanger resin. G-Trap™ DEAE Agarose Fast Flow and G-Trap™ Q Agarose Fast Flow contains anion exchanger resin.

G-Trap™ CM Agarose Fast Flow resin is a weak cation exchanger composed of highly crosslinked 6% agarose beads coupled with carboxymethyl (CM) weak cation exchange groups (Table 2).

G-Trap™ SP Agarose Fast Flow resin is a strong cation exchanger composed of highly crosslinked 6% agarose beads coupled with sulphopropyl (SP) strong cation exchange groups (Table 2).

G-Trap™ DEAE Agarose Fast Flow resin is a weak anion exchanger composed of highly crosslinked 6% agarose beads coupled with diethylaminoethyl (DEAE) weak anion exchange groups (Table 3).

G-Trap™ Q Agarose Fast Flow resin is a strong anion exchanger composed of highly crosslinked 6% agarose beads coupled with quaternary ammonium (Q) strong anion exchange groups (Table 3).

G-Trap™ Ion Exchange Agarose Fast Flow Columns are composed of highly crosslinked 6% agarose that enable excellent flow properties and high loading capacities (Table 2 and Table 3).

ITEM(S) SUPPLIED

Description	Cat. # 786-1018
G-Trap™ CM Agarose Fast Flow, 1 ml	1 column
G-Trap™ SP Agarose Fast Flow, 1 ml	1 column
G-Trap™ DEAE Agarose Fast Flow, 1 ml	1 column
G-Trap™ Q Agarose Fast Flow, 1 ml	1 column

Connector supplied with the G-Trap™ Ion Exchange Agarose Fast Flow Column:
Stop plug female, 1/16": For sealing bottom of G-Trap™ Ion Exchange Agarose Fast Flow column. One stop plug female is supplied per column.

STORAGE CONDITIONS

G-Trap™ Ion Exchange Agarose Fast Flow Columns are shipped at ambient temperature. Upon arrival, store it refrigerated at 4°C- 30°C, DO NOT FREEZE. This product is stable for 1 year at 4°C-30°C. The resin in the column should be stored in 20% ethanol at 4°C-30°C after use.

SPECIFICATIONS

Table 1: G-Trap™ Ion Exchange Agarose Fast Flow column

Features	1 ml column
Column Volume	1 ml
Column Dimensions	0.7 x 2.5 cm
Column Hardware Pressure Limit	0.5 MPa
Column hardware	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™ Cation Exchanger Resin

	G-Trap™ CM Agarose Fast Flow	G-Trap™ SP Agarose Fast Flow
Matrix	Cross-linked agarose beads, 6%	
Bead size	45-165 µm	
Ligand	Carboxymethyl	Sulphopropyl
Ion Exchanger	Weak cation exchanger	Strong cation exchanger
Ionic Capacity	0.09-0.13 mmol (H^+)/ml	0.18-0.25 mmol (Na^+)/ml
Binding Capacity	50 mg lysozyme/ ml medium	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	
Recommended Flow rate	1 ml/ min and 5 ml/ min for 1 ml and 5 ml column respectively	
Maximum Pressure	0.3MPa	
Exclusion limit(globular proteins)	4×10^6	
Chemical Stability	Stable to all commonly used aqueous buffers:1 M NaOH, 8M urea, 8M guanidine hydrochloride, 70% ethanol	
Storage Conditions	4 to 30°C, 20% Ethanol	4 to 30°C, 20% Ethanol containing 0.2M sodium acetate

Table 3: G-Trap™ Anion Exchanger Resin

	G-Trap™ DEAE Agarose Fast Flow	**G-Trap™ Q Agarose Fast Flow**
Matrix	Cross-linked agarose beads, 6%	
Bead size	45-165 µm	
Ligand	Diethylaminoethyl	Quaternary ammonium
Ion Exchanger	Weak anion exchanger	Strong anion exchanger
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	
Recommended Flow rate	1 ml/ min and 5 ml/ min for 1 ml and 5 ml column respectively	
Maximum Pressure	0.3MPa	
Exclusion limit(globular proteins)	4×10^6	
Chemical Stability	Stable to all commonly used aqueous buffers:1M NaOH, 8M urea, 8M guanidine hydrochloride, 70% ethanol	
Storage Conditions	4 to 30°C, 20% Ethanol	

IMPORTANT INFORMATION

- If the proteins to be separated are stable below it pI a cation exchanger is used and if they are stable above its pI then an anion exchanger is used.
- One need to select appropriate buffer with appropriate ionic strength based upon strategy selected for separation of biomolecules. Buffers such as MES, HEPES, phosphate, maleic acid are used for cation exchange chromatography[1]. Buffers such as Tris, piperazine, diethanlamine, bis-Tris are used for anion exchange chromatography[1].
- Three commonly used strategy for purification of substances is as follows
  Binding and elution of all biomolecules in the sample: Choose appropriate start buffer depending upon cation or anion exchanger with low ionic strength which will enable binding of all the biomolecules and then elute either by gradient or step-wise elution using higher concentration of salt such as NaCl.
  Binding of only the target protein: Choose appropriate buffer with pH optimum for maximum binding of desired protein. In such start buffers salt are added at high concentration for high ionic strength to avoid binding of other molecules other than desired or target protein.
  Binding of the contaminants of the sample: Under this strategy, start buffer with pH and ionic strength is chosen in such a way that it promotes binding of all contaminants except the target protein which comes out purified in flow through.
- **Starting pH:** For cation exchangers starting pH should be at least 1 pH unit below the pI of protein/substance to be bound and for anion exchangers it should be at least 1 pH unit above the protein/substance to be bound.
- The G-Trap™ Ion Exchange Agarose Fast Flow Columns can be operated with syringe, peristaltic pump or a chromatography system.
- G-Trap™ columns cannot be opened or refilled.

ADDITIONAL ITEMS REQUIRED

- **Union 1/16" male/luer female connecto**r: For connecting a syringe to G-Trap™ HIC Column.
- **Start Buffer**: Depending upon the exchanger type cation or anion different buffers can be selected[1] such as MES, citric acid for cation exchange chromatography and piperazine for anion exchange chromatography. The starting buffer concentration is 20 mM.
- **Elution buffer**: such as 20 mM start buffer plus 1 M NaCl.
- 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 or salting out the samples.

PROTOCOL

Sample Preparation

1. The sample buffer composition should be adjusted to the start buffer composition. This can be achieved either by diluting the sample with binding buffer or G-Trap™ GT-600 Desalting Columns (Cat. # 786-1023) or SpinOUT™ GT-600 (Cat. # 786-170).
2. Remove the particulates from sample either by centrifugation or filter through 0.45 µm filter.

Purification

1. Fill the syringe or the pump tubing with binding buffer before connecting to the G-Trap™ column to avoid introducing air into the column.
2. Remove the stopper and connect the column to syringe or pump tubing with the luer connector.
3. Remove the snap-off end of the column and wash the column with 10 CV (column volumes) of start buffer at 1ml/min or 5ml/ min for 1 ml and 5 ml G-Trap™ columns respectively.
4. Add sample to the column followed by washing the column with start buffer (5 to 10 CV) until no protein appears in the wash fractions collected. This can be monitored by measuring absorbance of collected fractions at 280 nm.
5. Elute with 5 to 10 CV of elution buffer.
   NOTE: Elution can be stepwise or gradient depending upon which is efficient for the separation of desired proteins/substances.
6. The purified fractions can be desalted using G-Trap™ GT-600 Desalting Columns (Ex:Cat. # 786-1023).
   NOTE: *The flow rate of the column should be maintained otherwise it may damage the column.*
   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.*

Column regeneration

Wash the column with 5CV of elution buffer (high salt) followed by 10CV of start buffer. The column is ready for reuse

STORAGE

1. Wash the G-Trap™ CM Agarose Fast Flow, G-Trap™ DEAE Agarose Fast Flow and G-Trap™ Q Agarose Fast Flow columns with 5CV of 20% ethanol and store the column in 20% ethanol at 4°C-30°C.

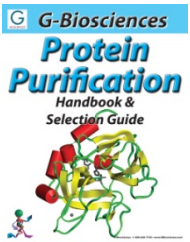
2. Wash the G-Trap™ SP Agarose Fast Flow column with 5CV of 20% ethanol containing 0.2M sodium acetate and store that column in 20% ethanol containing 0.2 M sodium acetate at 4°C-30°C.
   NOTE: *The bottom of the column is closed with the stop plug provided.*

REFERENCES

1. Lide, D. (2002).Handbook of chemistry and physics, 83rd edition, CRC.

RELATED PRODUCTS

Download our Protein Purification Handbook.



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

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