G-Trap™ IDA Agarose Fast Flow

Ni IDA and Co IDA Fast Flow columns (Cat. # 786-1019, 786-1020, 786-1021, 786-1022)

INTRODUCTION

G-Trap™ IDA Agarose Fast Flow columns are ready to use prepacked affinity columns used for purification of proteins based on immobilized metal ion affinity chromatography (IMAC). IMAC is based on interaction of cations of transition metals such as Ni^{2+}, Co^{2+} with protein residues histidine, cysteine and to some extent to tryptophan.

G-biosciences offer two types of G-Trap™ IDA Agarose Fast Flow columns namely G-Trap™ Co IDA Agarose Fast Flow and G-Trap™ Ni IDA Agarose Fast Flow. G-Trap™ Co IDA Agarose Fast Flow and G-Trap™ Ni IDA Agarose Fast Flow are specifically designed for purification of recombinant proteins fused with 6x histidine (6XHis) tag.

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.

The resin used in G-Trap™ IDA Agarose Fast Flow columns is highly cross-linked 6% agarose. Co^{2+} or Ni^{2+} are immobilized on cross-linked 6% agarose using iminodiacetic acid groups (IDA) which are covalently bound to resin (Table 2). Ni^{2+} IDA has a higher binding affinity but lower specificity compared to Co^{2+} IDA.

ITEM(S) SUPPLIED

Cat. #	Description	Size
786-1019	G-Trap™ Co IDA Agarose Fast Flow, 1 ml	5 columns
786-1020	G-Trap™ Co IDA Agarose Fast Flow, 5 ml	5 columns
786-1021	G-Trap™ Ni IDA Agarose Fast Flow, 1 ml	5 columns
786-1022	G-Trap™ Ni IDA Agarose Fast Flow, 5 ml	5 columns

Connector supplied with the G-Trap™ IDA Agarose Fast Flow Column:

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

STORAGE CONDITIONS

G-Trap™ IDA Agarose Fast Flow columns are shipped at ambient temperature. Upon arrival, store them at 4°C to 30°C, DO NOT FREEZE. This product is stable for 1 year at 4°C to 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™ IDA Agarose Fast Flow 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™ IDA Agarose Fast Flow resin

	G-Trap™ Ni IDA Agarose Fast Flow	G-Trap™ Co IDA Agarose Fast Flow
Matrix	Cross-linked agarose beads, 6%	
Mean particle size	90 µm	
Chelating Agent	Iminodiacetic acid	
Active group	Ni^{2+}	Co^{2+}
Metal ion density	20-40 µmol /ml	
Binding Capacity	5-10mg His-tagged protein/ml medium	
pH stability Working Range	3-12	
pH stability Cleaning-in-Place (CIP)	2-14	
Maximum Flow Velocity	450 cm/h	
Recommended Flow rate	1 ml/min and 5 ml/min for 1 ml and 5 ml column respectively	
Exclusion limit(globular proteins)	4×10^6	
Chemical Stability	Stable to all commonly used aqueous buffers: 8 M urea, 6 M guanidine hydrochloride	
Storage Conditions	2 to 8°C, 20% Ethanol	

IMPORTANT INFORMATION

- The G-Trap™ IDA Agarose Fast Flow column 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: For connecting a syringe to G-Trap™ IDA Agarose column.
- Binding/wash buffer such as 20 mM sodium phosphate, 0.5M NaCl, 20-40 mM imidazole, pH:7.4
- Elution buffer: ex. 20 mM sodium phosphate, 0.5M NaCl, 0.5 M imidazole, pH:7.4
- 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 after elution to remove imidazole from the sample.

PROTOCOL OPTIMIZATION

Equilibration and binding

Equilibration or start buffer is chosen based on properties of the protein to be purified and the type of chelate used. The most frequently used buffers are 50 mM acetate or 10 to 150 mM phosphate. The pH of the binding buffers is generally neutral (pH: 7.0 to 8.0) but may vary over range 5.5-8.5 depending upon the protein to be purified. To avoid ionic interaction 0.15-0.5 M NaCl can be added to the binding buffers. In some cases to avoid non-specific binding of proteins to the IMAC column, 10-40 mM imidazole can be added to binding buffer/start buffer. If the flow rate is decreased, then binding of protein to the column is enhanced.

Sample preparation

If the target His-tagged protein is soluble in cytoplasm, then dilute the sample in start buffer or perform buffer exchange using G-Trap™ GT-600 Desalting Columns (Cat. # 786-1023). If target His-tagged protein is present in inclusion bodies, then solubilize it with 8 M urea or 6 M guandine hydrochloride. However denaturants like urea and guanidine hydrochloride leads to complete denaturation of proteins. Protein can be refolded after elution on column but the refolding is protein specific.

Elution

Elution of the target protein can be achieved in several ways, including lowering pH, high concentration of imidazole or use of chelating agents like EDTA in the buffer.

The recommended elution buffer contains 0.5 M imidazole. It is also possible to use concentration gradient of 0-0.5 M imidazole to purify target protein.

Decreasing the pH of the elution buffer also allows elution of protein. Lower than pH 4 can result in stripping off of metal ions.

PROTOCOL

Sample Preparation

1. The sample buffer composition should be adjusted to the binding 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 5 CV (column volumes) of binding buffer at 1ml/min or 5ml/ min for 1 ml and 5 ml G-Trap™ IDA Agarose Fast Flow column respectively.
   NOTE: *Check the protocol optimization section for choice of binding and elution buffers.*
4. Add the pretreated sample to the column.
5. Wash the column with binding buffer till the effluent show baseline absorbance at 280 nm (10-15 CV).
6. Elute the target protein with elution buffer either by stepwise elution or by linear gradient method. For stepwise elution 5CV of elution buffer is sufficient. Linear gradient would require over 20CV of elution buffer and this method separate proteins with similar binding strengths.
7. Remove the imidazole from eluted fractions with G-Trap™ GT-600 Desalting Column (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

1. Wash the G-Trap™ IDA Agarose Fast Flow column with 5CV of stripping buffer removes the metal ions from agarose.
   NOTE: *Stripping buffer: 20mM sodium phosphate, 50 mM EDTA, 0.5 M NaCl.*
2. Wash the column with 5 CV of distilled water.

3. **Removal of proteins bound by ionic interaction:** Remove the ionic bound proteins from the column with several washes (10-15CV) with 1.5M NaCl solution for 20 minutes. After this wash the column with 5CV of distilled water
   Precipitated proteins removal: Wash in batch for atleast 2 hrs with 1 M NaOH. Remove NaOH with 10CV of distilled water.
   NOTE: *Long contact time of resin with NaOH enables inactivation of endotoxins.*
   Removal of hydrophobically bound proteins: Wash the column with 10 CV of 30% isopropanol for 20 minutes. After this wash the column with 10 CV of distilled water.
4. Alternatively, wash in batch for 2 hrs with 2CV of 0.5% non-ionic detergent in 0.1 M acetic acid. Remove the detergent with 5 CV of 70% ethanol followed washing the column with 10 CV distilled water.
5. Recharge the column using either Ni^{2+} or Co^{2+} with 5CV of 0.1M $NiSO_4.6H_2O$ or 5CV of 0.1 M cobalt (II) sulfate heptahydrate respectively.
6. Wash out excess metal ions with 5CV of distilled water.
7. Wash with 5CV of binding buffer before loading sample to the column.

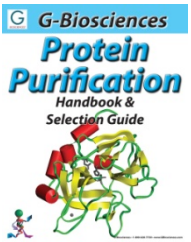
STORAGE

Wash the columns with 5CV of 20% ethanol and store the column in 20% ethanol at 4°C to 30°C.

NOTE: *The bottom of the column is closed with the stop plug provided.*

RELATED PRODUCTS

Download our Protein Purification Handbook.



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

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