G-Trap™ rProtein A FF

(Cat. # 786-1029, 786-1030, 786-1031, 786-1032)

INTRODUCTION

G-Trap™ rProtein A FF are prepacked ready to use columns for purification of antibodies. The column is packed with fast flow resin, which is covalently coupled to recombinant Protein A.

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

Recombinant Protein A is coupled covalently by proprietary coupling method to highly crosslinked 6% agarose.

The recombinant Protein A, produced in E. coli, has been genetically engineered to enhance its binding capacity to the antibodies

G-Trap™ rProtein A FF is used for the isolation and purification of a wide variety of immunoglobulin Gs from a variety of species and variety of samples such as monoclonal or polyclonal sera, ascites, cell culture supernatants etc. Check the appendix for relative binding affinities of recombinant Protein A and recombinant G to the antibodies.

G-Trap™ rProtein A FF has high binding efficiency of > 40mg human IgG/ml resin. The binding efficiency may vary depending upon the type of sample, concentration of sample and the column flow rate when sample was applied to the column.

The key characteristics of the recombinant Protein A resin or matrix used in G-Trap™ rProtein A FF are listed in Table 2

ITEM(S) SUPPLIED

Cat. #	Description	Size
786-1029	G-Trap™ rProtein A FF, 1 ml	5 columns
786-1030	G-Trap™ rProtein A FF, 5 ml	1 column
786-1031	G-Trap™ rProtein A FF, 1 ml	2 columns
786-1032	G-Trap™ rProtein A FF, 5 ml	5 columns

Connectors supplied with the G-Trap™ rProteinA FF:

1. Union 1/16” male/luer female (1): For connecting a syringe to G-Trap™ rProtein A FF column.
2. Stop plug female, 1/16”: This connector is for sealing bottom of G-Trap™ rProtein A FF G column. Two stop plugs female are supplied per column.
   NOTE: *Make sure the connectors are tightly connected to the column to avoid any leakage.*

STORAGE CONDITIONS

G-Trap™ rProtein A FF is 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™ rProtein A FF 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™ rProtein A FF resin

Mean particle size	90 µM
Bead Structure	Highly cross-linked 6% agarose
Ligand	Recombinant Staphylococcal Protein A from E.coli
Binding capacity	>40 mg Human IgG/ ml resin
Recommended working flow rate	1 ml/min and 5 ml/min for 1 ml and 5 ml column respectively.
Chemical Stability	Stable in all commonly used buffers
pH stability	Long term: pH 3-9, short term pH 2-9 **NOTE***: Long term refers to stability of matrix over long period without any adverse affects on functioning of column. Short term refers to when buffers with mentioned pH are used for short interval like for regeneration of resin, cleaning –in-place procedures* **NOTE:** *Although pH lower than 3 is suitable for eluting some strongly bound IgGs, it may lead to hydrolysis of the ligand*
Storage	2°C to 8°C in 20% ethanol

IMPORTANT INFORMATION

- Protein A can bind IgGs over wide range of pH, thus one can select from wide range of pH depending upon downstream applications of the purified IgGs. Since G-Trap™ rProtein A FF resin is stable in all of the commonly used buffers, Tris or phosphate buffers can be used for binding and washing as per requirement.
- Since IgGs are eluted at lower pH of around 2.5 or 3, it can damage the purified IgGs. Therefore it is recommended to add 50 to 200 µl of 1 M Tris-HCl pH 9, so that the final pH of the eluted fraction of IgGs is near neutral pH.
- The G-Trap™ rProtein A FF column can be operated with syringe, peristaltic pump or a chromatography system.
- G-Trap™ columns cannot be opened or refilled.

ADDITIONAL ITEMS REQUIRED

- Binding/wash buffer such as 20 mM sodium phosphate, pH 7.0
  NOTE: *Tris-HCl can also be used for binding, depending upon downstream application*
- Elution buffer: 0.1 M glycine-HCl, pH2.7 or 0.1 M sodium citrate, pH3-6
- Neutralization Buffer: 1 M Tris-HCl, pH9.0
- 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 IgGs .

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 provided along with the G-Trap™ rProtein A FF column.
3. Remove the snap-off end of the column and wash the column with 10 CV (column volumes) of binding 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 binding 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 the bound IgGs with 2 to 5 CV of elution buffer.
6. Identify the eluent fractions IgGs either by suitable protein assay such as NI-Protein Assay (Cat. # 786-005) or by ELISA.
7. Depending upon downstream application a buffer exchange can be performed with eluted IgGs using G-Trap™ GT-600 Desalting Columns (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.

STORAGE AND REUSE

1. G-Trap™ rProtein A FF can be re-used; however, it is preferred that they are used for same sample type to avoid any cross-contamination especially of monoclonal antibodies.
2. Wash the columns with 5CV of 20% ethanol and store the column in 20% ethanol at 4°C.
   NOTE: *The bottom of the column is closed with the stop plug provided.*

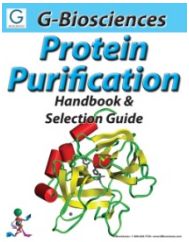
APPENDIX

Table1: Relative affinity of Protein A and Protein G for Immunoglobulins

Species	Antibody Class	Protein A	Protein G
Mouse	Total IgG	++++	++++
	IgG_1	+	+++
	IgG_{2a}	++++	++++
	IgG_{2b}	++++	++++
	IgG_3	+++	+++
Human	Total IgG	++++	++++
	IgG_1	++++	++++
	IgG_2	++++	++++
	IgG_3	+	++++
	IgG_4	++++	++++
Rat	Total IgG	+	++
	IgG_1	-	+
	IgG_{2a}	-	++++
	IgG_{2b}	-	++
	IgG_{2c}	++	+++
Hamster	Total IgG	++	++
Guinea Pig	Total IgG	++++	++
Rabbit	Total IgG	++++	+++
Horse	Total IgG	++	++++
Cow	Total IgG	++	++++
Pig	Total IgG	+++	++
Sheep	Total IgG	+	++
Goat	Total IgG	+	++
Chicken	Total IgG	-	-

RELATED PRODUCTS

Download our Protein Purification Handbook.



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

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