rProtein A **GraviTrap**
Protein G **GraviTrap**
rProtein A/Protein G **GraviTrap**

Instructions

cytiva.com

28987231 AB

Table of Contents

1 Introduction

About

rProtein A GraviTrap™ contains:

- 10 × 1 ml prepacked rProtein A GraviTrap columns
- 10 bottom caps
- Instructions for use

Protein G GraviTrap contains:

- 10 × 1 ml prepacked Protein G GraviTrap columns
- 10 bottom caps
- Instructions for use

rProtein A/Protein G GraviTrap contains:

- 10 × 1 ml prepacked rProtein A/Protein G GraviTrap columns
- 10 bottom caps
- Instructions for use

Purpose

Prepacked rProtein A GraviTrap, Protein G GraviTrap and rProtein A/Protein G GraviTrap columns are designed for single-step, manual, gravity-flow, purification of antibodies from different species.

rProtein A GraviTrap, Protein G GraviTrap and rProtein A/ Protein G GraviTrap provide 10 prepacked columns each, allowing multiple samples to be run simultaneously in a convenient gravity column format. Each column contains 1 ml bed volume and can bind milligram amounts of antibodies. All the three products are delivered in a package that can be converted into a column stand (Workmate) for a simpler purification. The plastic tray in the package can be used to collect liquid waste.

Connecting LabMate reservoir to the column increases convenience when handling volumes above 10 ml. This raises the loading capacity volume to approximately 35 ml in one go (see *Chapter 7 Ordering information, on page 12*).

Important

Read these instructions carefully before using the products.

Intended use

The products are intended for research use only, and shall not be used in any clinical or *in vitro* procedures for diagnostic purposes.

Safety

For use and handling of the products in a safe way, refer to the Safety Data Sheets.

Storage

Store at +2°C to +8°C. Do not freeze.

2 Principle

rProtein A GraviTrap, Protein G GraviTrap and rProtein A/ Protein G GraviTrap are gravity-flow columns prepacked with 1 ml of rProtein A Sepharose™ FF, Protein G Sepharose 4 FF and 50% rProtein A Sepharose FF and 50% Protein G Sepharose 4 FF, respectively, which have high affinity for antibodies from various species.

Protein G and protein A are bacterial proteins from Group G *Streptococci* and *Staphylococcus aureus*, respectively, and have high affinity for the Fc region of polyclonal and monoclonal IgG-type antibodies that forms the basis for purification of IgG and IgG fragments.

3 Antibody binding to protein A and protein G

The binding strengths of protein A and protein G for immunoglobulins depend on the source species and subclass of the particular immunoglobulin.

Table 1. Relative binding strengths for protein A and protein G

Species	Subclass	Protein A binding	Protein G binding
Human	IgA	variable	-
	IgD	-	-
	IgD	-	-
	IgG_1	++++	++++
	IgG_2	++++	++++
	IgG_3	-	++++
	IgG_4	++++	++++
	IgM	variable	-
Avian egg yolk	IgY	-	-
Cow		++	++++
Dog		++	+
Goat		-	++
Guinea pig	IgG_1	++++	++
	IgG_2	++++	++
Hamster		+	++
Horse		++	++++
Koala		-	+
Llama		-	+
Monkey (rhesus)		++++	++++
Mouse	IgG_1	+	++++
	IgG_{2a}	++++	++++
	IgG_{2b}	+++	+++
	IgG_3	++	+++
	IgM	variable	-
Pig		+++	+++

Rabbit		++++	+++
Rat	IgG_1	-	+
	IgG_{2a}	-	++++
	IgG_{2b}	-	++
	IgG_3	-	++
Sheep		+/-	++

++++ = strong binding, ++ = medium binding, - = weak or no binding

4 Advice on handling

Optimization of parameters

The protocol recommended in this instruction (see *Chapter 5 Antibody purification protocol, on page 9*) is suitable for purification of most antibodies. However, some parameters for antibody purification may require optimization to obtain the best result.

Examples of parameters which may require optimization are:

- Choice of buffers
- Sample pre-treatment
- Amount of antibody to be purified
- Number of washes

Recommended buffers

Note: *Use high-purity water and chemicals for buffer preparation.*

Buffer	Composition
Binding/buffer	20 mM sodium phosphate, pH 7.0
Elution buffer	0.1 M glycine-HCl, pH 2.7
Neutralizing buffer	1 M Tris-HCl, pH 9.0

- Recommended buffers can be prepared easily using the 10 × stock solutions of binding and elution buffers supplied with the Ab Buffer Kit (see *Chapter 7 Ordering information, on page 12*).
- rProtein A GraviTrap, Protein G GraviTrap and rProtein A/Protein G GraviTrap bind immunoglobulins over a wide pH range and thus permit the use of a variety of buffers. Generally, rProtein A GraviTrap, Protein G GraviTrap and rProtein A/Protein G GraviTrap bind IgG with a strong affinity at pH 7.0.
- Different immunoglobulins elute at different pH values depending on the subclass and the species from which they originate. For antibodies sensitive to low pH, optimize elution by determining the highest pH that allows efficient elution.

Sample pre-treatment

- Check the pH of the sample, and adjust, if necessary, before applying the sample to the column. The pH of the sample should be equal to the pH of the binding buffer. pH can be adjusted by either diluting the sample with binding buffer or by buffer exchange using PD-10 Desalting Columns or HiTrap™ Desalting columns (see *Chapter 7 Ordering information, on page 12*).
- Clarify the sample before applying it to the medium.

5 Antibody purification protocol

This protocol is suitable for rProtein A GraviTrap, Protein G GraviTrap and rProtein A/Protein G GraviTrap columns described in *Chapter 2 Principle, on page 5*.

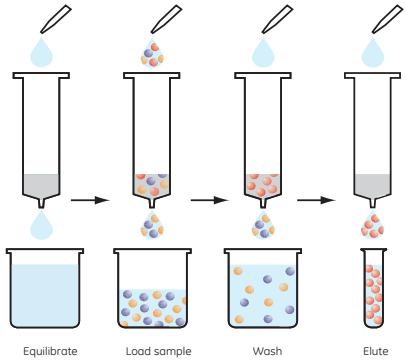

Fig 1. Purifying antibodies with rProtein A GraviTrap, Protein G GraviTrap and rProtein A/Protein G GraviTrap columns is a simple four-stage procedure.

Step	Action
1	**Column preparation** **a.** Cut off the bottom tip and remove the top cap. **b.** Pour off the column storage solution and place the column in the Workmate column stand. If needed, mount LabMate on top of the column.
2	**Equilibration.** Equilibrate the column with 10 ml of binding buffer.

Step	Action
3	**Sample application.** After equilibration, add the sample (see *Chapter 4 Advice on handling, on page 7*). A volume of 1 to 20 ml is recommended. If the sample volume is less than 1 ml, dilute to 1 ml with binding buffer.
4	**Washing.** Add 15 ml binding buffer.
5	**Elution** **a.** Add 3 to 5 ml of elution buffer. **b.** Collect the elution fraction. The collected elution fraction contains the purified protein. ***Note:*** *As a safety measure to preserve the activity of acid-labile IgGs, addition of 1 M Tris-HCl, pH 9.0, to the tubes used for collecting antibody-containing fractions (60 to 200 µl/ml eluted fraction) is recommended. In this way, the final pH of the sample will be approximately neutral.* ***Tip:*** *The eluted fractions can be buffer exchanged using PD-10 Desalting columns or HiTrap Desalting columns (see Chapter 7 Ordering information, on page 12).*
6	**Regeneration** **a.** After elution, regenerate the column by washing it with 5 to 10 ml of binding buffer. The column is now ready for a new purification.

Step	Action
b.	Depending on the nature of the sample, rProtein A GraviTrap, Protein G GraviTrap and rProtein A/ Protein G GraviTrap columns may be reused up to five times consecutively. Reuse of the columns should only be considered when processing identical samples to avoid cross-contamination.

6 Characteristics

Table 2. rProtein A GraviTrap columns

Column material	Polypropylene barrel, polyethylene frits
Matrix	Highly cross-linked agarose, 4%
Medium	rProtein A Sepharose Fast Flow
Ligand	Recombinant protein A
Bed volume	1 ml
Binding capacity	~ 50 mg human IgG /ml medium
Particle size	90 µm
Working temperature	Room temperature
Storage solution	20% ethanol
Storage temperature	+2°C to +8°C

Table 3. Protein G GraviTrap columns

Column material	Polypropylene barrel, polyethylene frits
Matrix	Highly cross-linked agarose, 4%
Medium	Protein G Sepharose 4 Fast Flow
Ligand	Recombinant protein G
Bed volume	1 ml
Binding capacity	~ 20 mg human IgG /ml medium
Particle size	90 µm
Working temperature	Room temperature
Storage solution	20% ethanol

Storage temperature	+2°C to +8°C

Table 4. rProtein A/Protein G GraviTrap columns

Column material	Polypropylene barrel, polyethylene frits
Matrix	Highly cross-linked agarose, 4%
Medium	rProtein A Sepharose Fast Flow and Protein G Sepharose 4 Fast Flow
Ligand	Recombinant protein A and protein G
Bed volume	1 ml
Binding capacity	~ 35 mg human IgG /ml medium
Particle size	90 µm
Working temperature	Room temperature
Storage solution	20% ethanol
Storage temperature	+2°C to +8°C

7 Ordering information

Products	Quantity	Product code
rProtein A GraviTrap	10 × 1 ml	28985254
Protein G GraviTrap	10 × 1 ml	28985255
rProtein A/Protein G GraviTrap	10 × 1 ml	28985256

Related products	Quantity	Product code
Ab Buffer Kit	1	28903059
LabMate PD-10 Buffer Reservoir	10	18321603
Disposable PD-10 Desalting Columns	30	17085101
HiTrap Desalting	5 × 5 ml	17140801
Antibody Purification Handbook	1	18103746

Page intentionally left blank

cytiva.com/sampleprep

For local office contact information, visit cytiva.com/contact

28987231 AB V:5 03/2021