rProtein A GraviTrap™
Protein G GraviTrap
rProtein A/Protein G GraviTrap

rProtein A GraviTrap, Protein G GraviTrap, and rProtein A/Protein G GraviTrap are prepacked gravity-flow columns designed for fast and efficient manual purification of monoclonal and polyclonal antibodies, antibody fragments from cell culture supernatant, and biological fluids. The antibodies are simply captured with high specificity on protein A and protein G ligands in gravity-flow columns. You do not need any other instrument with this protocol because the entire process relies on the flow of gravity. The yield varies from 20 to 50 mg of purified antibodies depending on the ligand you use. The columns are reusable up to five times.

rProtein A GraviTrap, Protein G GraviTrap, and rProtein A/Protein G GraviTrap deliver:

- **Convenience and reliability**—prepacked gravity-flow columns provide convenience, save time, and increase the reliability of your results
- **Speed and simplicity**—purification of antibodies in 30 min allows you to quickly obtain and store your target protein in order to avoid the inherent dangers of protein instability and degradation associated with slow and long purification runs
- **High purity and recovery**—purity > 95% in one step and recovery typically 70% to 80%
- **High binding capacity**—rProtein A GraviTrap ~ 50 mg human IgG/column, Protein G GraviTrap ~ 20 mg human IgG/column, and rProtein A/Protein G GraviTrap ~ 35 mg human IgG/column

The ready-to-use columns are prepacked with 1 ml of rProtein A Sepharose™ Fast Flow, Protein G Sepharose 4 Fast Flow, and a mixture of 50% rProtein A Sepharose Fast Flow and 50% Protein G Sepharose 4 Fast Flow. The main characteristics of the columns are described in Table 1.

Fig 1. rProtein A GraviTrap, Protein G GraviTrap, and rProtein A/Protein G GraviTrap prepacked gravity flow columns provide simple manual purification of antibodies from cell culture.

Table 1. rProtein A, Protein G, and rProtein A/Protein G GraviTrap column characteristics

Column material	Polypropylene barrel, polyethylene frits
Matrix	Highly cross-linked agarose, 4%
Medium	rProtein A Sepharose Fast Flow, Protein G Sepharose 4 Fast Flow or a mixture of 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	rProtein A Sepharose Fast Flow ~ 50 mg human IgG /ml medium, Protein G Sepharose 4 Fast Flow ~ 20 mg human IgG /ml medium, rProtein A/Protein G Sepharose 4 Fast Flow ~ 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

Antibody binding to Protein A and Protein G

The binding strengths of protein A and protein G for immunoglobulins (IgG) depend on the source species and subclass of the particular immunoglobulin (Table 2). You may begin with rProtein A/Protein G GraviTrap if the binding strength of the target immunoglobulin is unknown.

Table 2. 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

Simple purification of antibodies in 30 min

The purification of immunoglobulins with rProtein A GraviTrap, Protein G GraviTrap, and rProtein A/Protein G GraviTrap comprises equilibration, sample application, washing and elution steps (Fig 2). The duration of a single purification run is about 30 min (depending on the volume and the viscosity of your sample). You can perform antibody purifications with a wide choice of buffers because rProtein A GraviTrap, Protein G GraviTrap, and rProtein A/Protein G GraviTrap have high affinities for a broad range of immunoglobulins at about pH 7.0. To attain effective binding, the pH of your sample should be the same as that of the binding buffer prior to sample application.

IgG elution occurs by lowering the pH. Different immunoglobulins elute at different pH values depending on the subclass and the species from which they originate. We recommend that you add neutralizing buffer to the column eluate in order to preserve the activity of acid-labile IgGs.

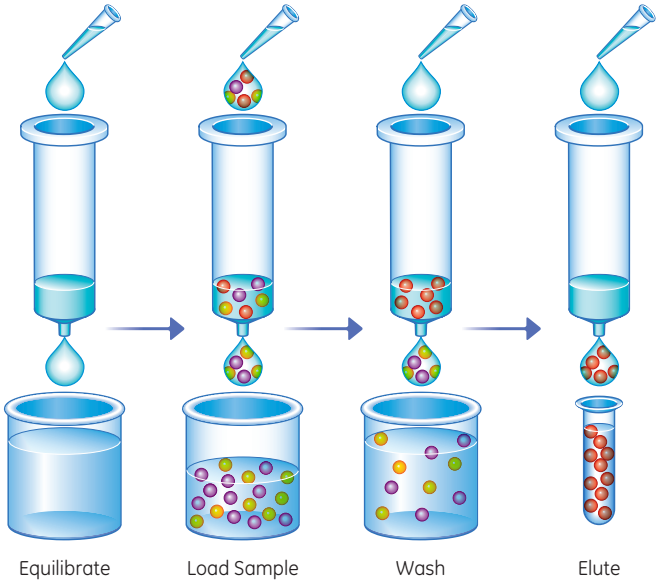

Fig 2. A fast and simple four-stage process for purifying immunoglobulins on rProtein A GraviTrap, Protein G GraviTrap, and rProtein A/Protein G GraviTrap columns.

Complementary products provide speed and convenience

The use of complementary products like Workmate, LabMate™, and Ab Buffer Kit with rProtein A GraviTrap, Protein G GraviTrap, and rProtein A/Protein G GraviTrap columns further enhances the versatility of the gravity-flow antibody purification method described in this data file (Fig 3).

Workmate and LabMate

We provide rProtein A GraviTrap, Protein G GraviTrap, and rProtein A/Protein G GraviTrap columns in a format that you can convert into a column stand (Workmate). The plastic tray in the package can be used to collect liquid waste. If you need to work with a sample volume that is > 10 ml, connecting a LabMate buffer reservoir to the column increases the loading capacity to about 35 ml.

Ab Buffer Kit

You can eliminate the tedious step of buffer preparation when you use the Ab Buffer Kit with rProtein A GraviTrap, Protein G GraviTrap or rProtein A/Protein G GraviTrap columns. The kit contains optimized buffers for each step (binding, elution, and neutralizing) of the purification of monoclonal and polyclonal IgG.

Fig 3. Workmate, LabMate, and Ab Buffer Kit complement rProtein A GraviTrap, Protein G GraviTrap, and rProtein A/Protein G GraviTrap columns, thus adding flexibility and versatility to the gravity-flow antibody purification method.

Reusability of GraviTrap columns

You can reuse the same GraviTrap column to purify the same antibody up to 5 times consecutively without having any adverse effect on the performance of the column. We performed five consecutive purification runs on three Protein G GraviTrap columns (Table 3) in order to test the reusability of the column. The IgG recovery was > 78% for all purification cycles (Fig 4), and IgG purity was > 95% based on SDS gel electrophoresis and analysis with ImageQuant™ TL software (data not shown).

Table 3. Experimental conditions for Protein G GraviTrap reusability study

Columns/media	Protein G GraviTrap
Sample	Human IgG spiked in *E. coli* lysate
Sample load	50% of theoretical binding capacity
Binding/wash buffer	20 mM sodium phosphate, pH 7.0
Elution buffer	0.1 M glycine-HCl, pH 2.7

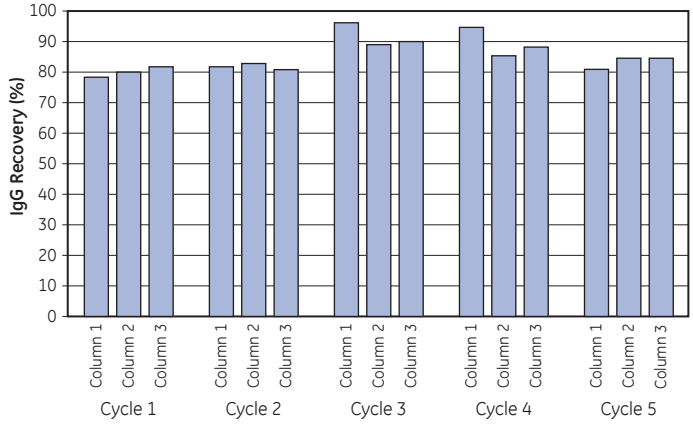

Fig 4. Five consecutive purification cycles on a Protein G GraviTrap column produced similar IgG recovery thus showing that the columns are highly reliable and reusable.

High repeatability of IgG purifications

We carried out a repeatability study by performing six replicate runs on each type of column (i.e., six each of rProtein A GraviTrap, Protein G GraviTrap, and rProtein A GraviTrap/ Protein G GraviTrap columns). The IgG recovery, calculated by absorbance measurements and extinction coefficient, was 72% to 84% depending on column type.

Table 4. Experimental conditions for a repeatability study on rProtein A GraviTrap, Protein G GraviTrap, and rProtein A GraviTrap/Protein G GraviTrap columns

Columns/media	rProtein A GraviTrap Protein G GraviTrap rProtein A GraviTrap/Protein G GraviTrap
Sample	Human IgG spiked in *E. coli* lysate
Sample load	50% of theoretical binding capacity
Binding/wash buffer	20 mM sodium phosphate, pH 7.0
Elution buffer	0.1 M glycine-HCl, pH 2.7

The results (Fig 5) show that the purification runs were highly repeatable with a relative standard deviation (RSD) of < 2% for the IgG recovery in all cases.

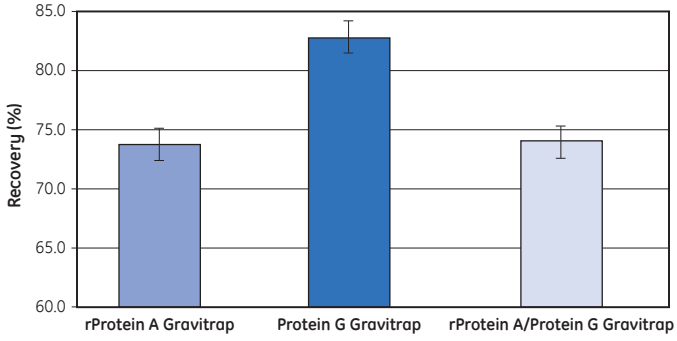

Fig 5. Six replicate runs on rProtein A Gravitrap, Protein G GraviTrap, and rProtein A/Protein G Gravitrap columns show that the purification runs were highly repeatable. The relative standard deviation (RSD) for the IgG recovery was < 2% for all column types. Y-error bars represent the standard error. Note that the values of the y-axis range from 60% to 85%.

Applications

Comparative purification of human monoclonal antibodies (MAb) with two different columns

In a comparative study, we used two rProtein A/Protein G GraviTrap and two Pierce™ Protein A/G Plus Agarose columns to purify human monoclonal antibodies from Chinese Hamster Ovary (CHO) cell cultivation media (Table 5). We determined the recovery and purity for each purification cycle with each of the columns. The purification runs were performed at the laboratories of GE Healthcare Life Sciences according to the instructions supplied with each column.

Table 5. Experimental conditions for human monoclonal antibody purification

Supplier	GE Healthcare	Thermo Scientific
Columns/media	rProtein A/Protein G GraviTrap	Protein A/G Plus Agarose
Binding capacity	~ 35 mg human IgG/ml medium	> 50 mg human IgG/ml medium
Sample load	70% of theoretical binding capacity	70% of theoretical binding capacity
Binding/wash buffer	20 mM sodium phosphate, pH 7.0	Protein A/G Binding Buffer, Prod # 54200 (Thermo Scientific)
Elution buffer	0.1 M glycine-HCl, pH 2.7	0.1 M glycine-HCl, pH 2.7

Simplicity and speed are very important factors in the use of gravity columns to purify antibodies because of the risk of protein instability and degradation. In this comparative study, the total purification time for the rProtein A/Protein G GraviTrap column was 26 min while that of the Pierce Protein A/G Plus A was 84 min. Thus the duration of the rProtein A/Protein G GraviTrap column was more than three times faster than that of Pierce Protein A/G Plus A.

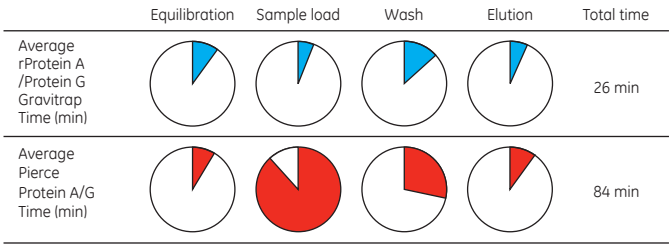

Fig 6. Average duration of protein purification cycles (two columns of each type) on rProtein A/Protein G GraviTrap and Pierce Protein A/G Plus Agarose columns. The speed of an antibody purification cycle with rProtein A/Protein G GraviTrap columns was more than three times faster than that of Pierce Protein A/G Plus Agarose columns.

In the use of gravity columns to purify antibodies, speed and simplicity cannot be attained at the expense of other crucial factors such as degree of recovery and purity. rProtein A/Protein G GraviTrap column produced significantly higher sample recovery than the corresponding Protein A/G Plus columns from Pierce (Fig 7).

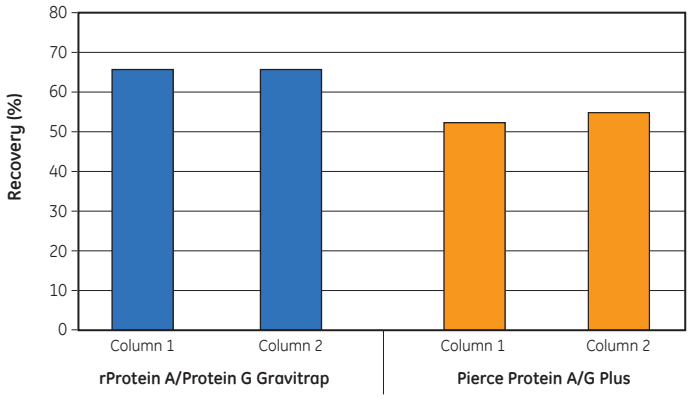

Fig 7. Recovery of human monoclonal antibody on rProtein A/Protein G GraviTrap and Pierce Protein A/G Plus columns.

Determination of MAb recovery via absorbance measurements and extinction coefficient was 67% with the rProtein A/Protein G GraviTrap columns, and 53% with the Pierce Protein A/G Plus columns. Protein pattern and purity were checked by SDS gel electrophoresis with Deep Purple™ Total Protein Stain (Fig 8).

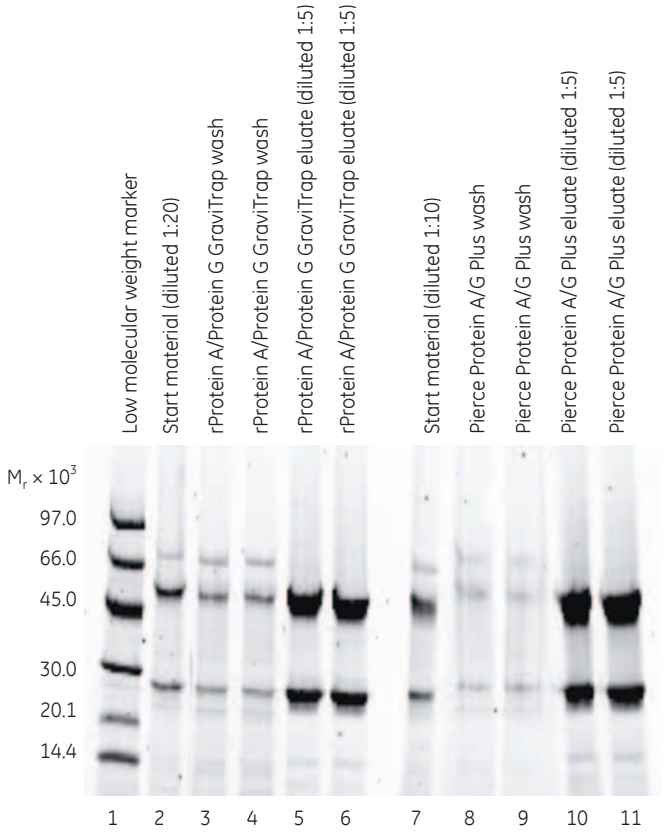

Fig 8. SDS-PAGE stained with Deep Purple Total Protein Stain (under reducing conditions) of fractions from the human monoclonal antibody purification on rProtein A/Protein G GraviTrap and Pierce Protein A/G Plus Agarose columns.

Comparative purification of rabbit serum IgG with 2 different columns

In another comparative study performed at the laboratories of GE Healthcare, we used 2 rProtein A GraviTrap and 2 Econo-Pac™ Protein A Affi-Gel™ columns from Bio-Rad™ to purify rabbit serum IgG (anti-goat) according to their manufacturers' instructions. The experimental conditions are summarized in Table 6.

Table 6. Experimental conditions for purification of rabbit serum IgG

Supplier	GE Healthcare	Bio-Rad
Columns/media	rProtein A GraviTrap	Econo-Pac Protein A Affi-Gel
Sample load	2 ml rabbit serum (diluted 1:1 with binding buffer)	2 ml rabbit serum (diluted 1:1 with binding buffer)
Binding/wash buffer	20 mM sodium phosphate, pH 7.0	Affi-Gel Protein A MAPS™II Binding Buffer (Kit #732-2020)
Elution buffer	0,1 M glycine-HCl, pH 2.7	Affi-Gel Protein A MAPSII Elution Buffer (Kit #732-2020)

Figure 9 shows the average duration of each individual purification step as well as the total purification time. The results show that the rProtein A GraviTrap column was more than three times faster than the Econo-Pac Protein A Affi-Gel.

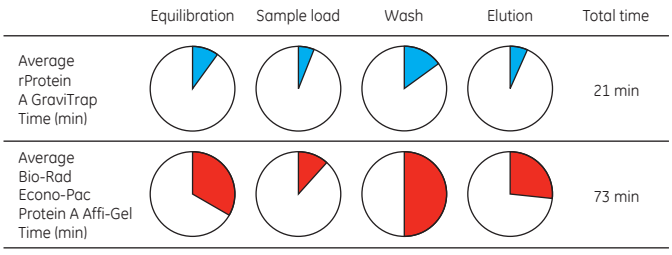

Fig 9. Average duration of protein purification cycles (2 columns of each type) on rProtein A GraviTrap and Bio-Rad Econo-Pac Protein A Affi-Gel columns. The speed of an antibody purification cycle with rProtein A columns was more than three times faster than that of Bio-Rad Econo-Pac Protein A Affi-Gel columns.

Despite the larger gel volume of the Bio-Rad Econo-Pac Protein A columns, Figure 10 shows that the binding capacity of rProtein A GraviTrap and Bio-Rad Econo-Pac Protein A Affi-Gel columns were similar. About 11 mg of purified rabbit IgG was obtained on each column in a single purification run.

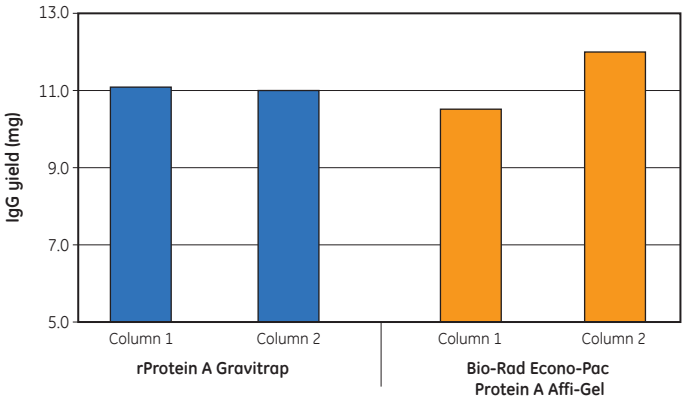

Fig 10. Purification of rabbit IgG on rProtein A GraviTrap and Bio-Rad Econo-Pac Protein A Affi-Gel columns. Each bar represents the amount of purified IgG from each column in a single purification run. We noticed a difference in binding capacity between the two Bio-Rad Econo-Pac Protein A columns.

Protein pattern and purity were checked via SDS gel electrophoresis stained with Deep Purple Total Protein Stain (Fig 11).

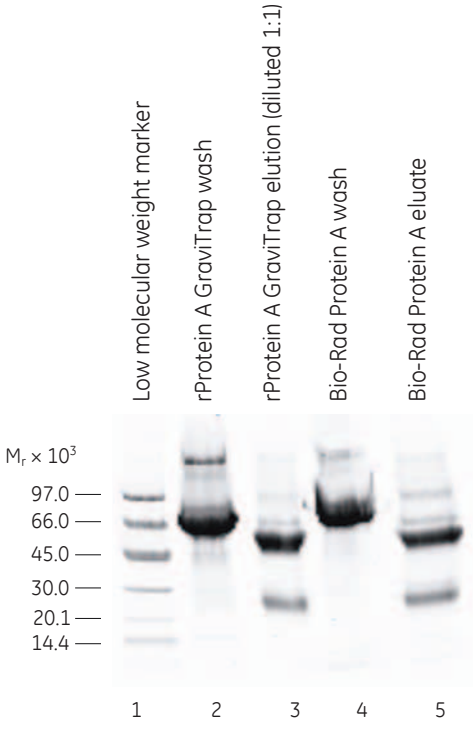

Fig 11. SDS-PAGE stained with Deep Purple Total Protein Stain (under reducing conditions) of eluted fractions from purification of rabbit IgG on rProtein A GraviTrap and Bio-Rad Econo-Pac Protein A Affi-Gel columns.

Ordering information

Products	Quantity	Code No.
rProtein A GraviTrap	10 × 1 ml	28-9852-54
Protein G GraviTrap	10 × 1 ml	28-9852-55
rProtein A/Protein G GraviTrap	10 × 1 ml	28-9852-56

Related products	Quantity	Code No.
Ab Buffer Kit	1	28-9030-59
LabMate PD-10 Buffer Reservoir	10 reservoirs	18-3216-03
Disposable PD-10 Desalting Columns	30 columns	17-0851-01
HiTrap Desalting 5× 5 ml	5 columns	17-1408-01

For your local office contact information, visit
www.gelifesciences.com/contact

GE Healthcare Bio-Sciences AB
Björkgatan 30
751 84 Uppsala
Sweden

www.gelifesciences.com/sampleprep

imagination at work

GE Healthcare UK Ltd, Amersham Place
Little Chalfont, Buckinghamshire, HP7 9NA
UK

GE Healthcare Bio-Sciences Corp
800 Centennial Avenue, P.O. Box 1327, Piscataway, NJ 08855-1327
USA

GE Healthcare Europe GmbH
Munzinger Strasse 5, D-79111 Freiburg,
Germany

GE Healthcare Japan Corporation
Sanken Bldg., 3-25-1, Hyakunincho, Shinjuku-ku, Tokyo 169-0073
Japan

28-9921-04 AA 03/2011