Protein A Mag Sepharose™ Xtra
Protein G Mag Sepharose Xtra

Protein A Mag Sepharose Xtra and Protein G Mag Sepharose Xtra products are magnetic beads designed for efficient, high capacity small-scale purification/screening of monoclonal and polyclonal antibodies from various species. Antibodies are captured with high specificity on protein A and protein G ligands on Mag Sepharose followed by the collection of the beads with a magnetic device. Magnetic beads simplify sample handling in small-scale purification.

Protein A Mag Sepharose Xtra and Protein G Mag Sepharose Xtra deliver:

- High-capacity small-scale purification of antibodies from various species
- High purity and yield
- Easy parallel screening of antibodies with high repeatability
- Scalability: Simple capture of antibodies from small or large sample volumes (low-microliter to high-milliliter scale)

The beads are each available in two pack sizes: 2 × 1 ml 10% medium slurry and 5 × 1 ml 10% medium slurry—a 1 ml 10% medium slurry is the same as a 100 µl sedimented medium. A 1 ml medium slurry is sufficient for 10 purification runs according to the recommended protocol. Together with MagRack 6, a separation tool for handling the beads in microcentrifuge tubes, up to six samples can be processed in parallel. You can easily screen a large number of samples in parallel with high throughput on a robotic device.

Reliable capture of antibodies

Protein A Mag Sepharose Xtra and Protein G Mag Sepharose Xtra are affinity chromatography media (resins) with protein A or protein G ligands that have high affinity for monoclonal or polyclonal antibodies. The binding strengths of protein A and protein G for immunoglobulins depend on the source species and subclass of the particular immunoglobulin. Table 1 shows the relative binding strengths of protein A and protein G.

Fig 1. Protein A Mag Sepharose Xtra and Protein G Mag Sepharose Xtra are designed for high capacity purification of antibodies from cell culture supernatant and biological fluids.

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

Simplified handling

The magnetic bead format has excellent properties for small-scale experiments. The high density of the beads allows rapid capture by magnetic devices while the visibility of the beads ensures reliable collection of the bound antibodies in the purification procedure. The products are provided with protocols optimized for antibody purification.

MagRack 6 enables preparation of up to six samples captured in 1.5 ml microcentrifuge tubes (Fig 2). When the tubes are placed in the rack, the magnetic beads are attracted to the magnet within a few seconds. This allows easy removal of the supernatant whereas the magnetic beads are left in the tube.

Fig 2. The high density of the beads allows rapid capture by MagRack 6 magnetic device.

Ab SpinTrap Buffer Kit can be used to eliminate time-consuming buffer preparations and this leads to fast and reproducible antibody purification. The characteristics of Protein A Mag Sepharose Xtra and Protein G Mag Sepharose Xtra are summarized in Table 2.

Antibody binding capacity—a benchmark analysis

A comparative benchmark analysis was performed at GE Healthcare Laboratories to investigate the binding capacity for antibodies using different magnetic beads products. We compared the performance of Protein A Mag Sepharose Xtra and Protein G Mag Sepharose Xtra with corresponding magnetic bead products from Millipore™, Invitrogen™, and Qiagen™ (Table 3). Human IgG and rabbit IgG were purified according to their manufacturer's instructions (Table 3). All the media were subjected to an overloading test (i.e., the load of IgG was above the capacity for each media).

Table 2. Characteristics of Protein A Mag Sepharose Xtra and Protein G Mag Sepharose Xtra

Protein A Mag Sepharose Xtra	
Matrix	Highly crosslinked spherical agarose (Sepharose) including magnetite
Medium	Protein A coupled NHS activated Mag Sepharose
Ligand	Protein A
Binding capacity	> 27 mg human IgG/ml medium
Particle size	37 to 100 µm
Working temperature	Room temperature
Storage solution	20% ethanol, 10% medium slurry
Storage temperature	+ 4°C to + 8°C

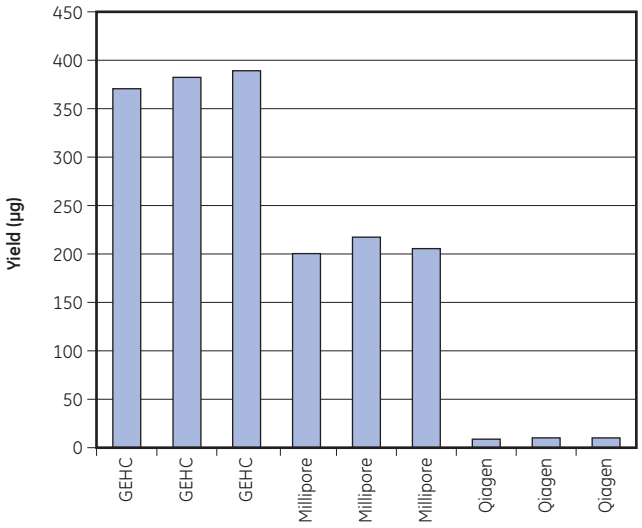
Protein G Mag Sepharose Xtra	
Matrix	Highly crosslinked spherical agarose (Sepharose) including magnetite
Medium	Protein G coupled NHS activated Mag Sepharose
Ligand	Protein G
Binding capacity	> 27 mg human IgG/ml medium
Particle size	37 to 100 µm
Working temperature	Room temperature
Storage solution	20% ethanol, 10% medium slurry
Storage temperature	+ 4°C to + 8°C

Table 3. Experimental conditions for protein A media and protein G media

Supplier	GE Healthcare (GEHC)	Millipore	Qiagen	Invitrogen
Chromatography media	Protein A Mag Sepharose Xtra and Protein G Mag Sepharose Xtra	PureProteome™ Protein A and PureProteome Protein G Magnetic Beads	BioMag™ Protein A and BioMag Protein G	Dynabeads™ Protein A and Dynabeads Protein G
Load	Overloaded	Overloaded	Overloaded	Overloaded
Binding/wash buffer	PBS (140 Mm NaCl, 2.7 mM KCl, 10 mM phosphate, pH 7.4)	PBS (140 mM NaCl, 2.7 mM KCl, 10 mM phosphate, pH 7.4)	100 mM Tris-HCl, 150 mM NaCl, pH 7.5	PBS (140 mM NaCl, 2.7 mM KCl, 10 mM phosphate, pH 7.4) including 0.02% Tween 20
Elution buffer	100 mM glycine, pH 2.8	200 mM glycine, pH 2.5	200 mM glycine, pH 2.5	100 mM glycine, pH 2.8

The results show that Protein A Mag Sepharose Xtra and Protein G Mag Sepharose Xtra have considerably higher binding capacities for human IgG than the corresponding products from Millipore and Qiagen. Approximately 350 to 400 µg of purified human IgG was obtained in a single run with Protein A Mag Sepharose Xtra and Protein G Mag Sepharose Xtra (Figures 3A and 3B).

A). Protein A magnetic beads



B). Protein G magnetic beads

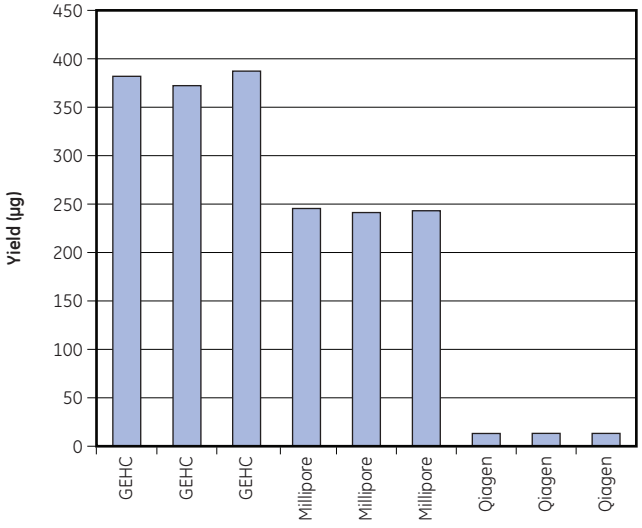
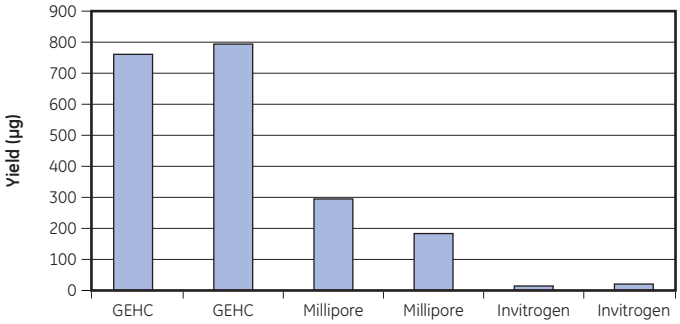


Fig 3. Purification of human IgG using different protein A and protein G magnetic beads. Each bar represents the amount of purified IgG obtained in a single purification run.

Protein A Mag Sepharose Xtra and Protein G Mag Sepharose Xtra produced higher binding capacities than the corresponding products from Millipore and Invitrogen during the purification of rabbit IgG. For example, a single purification run resulted in 700 to 800 µg purified rabbit IgG (Figures 4A and 4B).

A). Protein A magnetic beads



B). Protein G magnetic beads

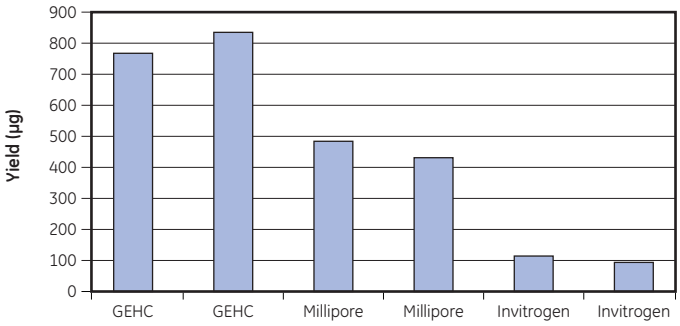


Fig 4. Purification of rabbit IgG using different protein A and protein G magnetic beads in a comparative performance evaluation. Each bar represents the amount of purified IgG obtained in a single purification run.

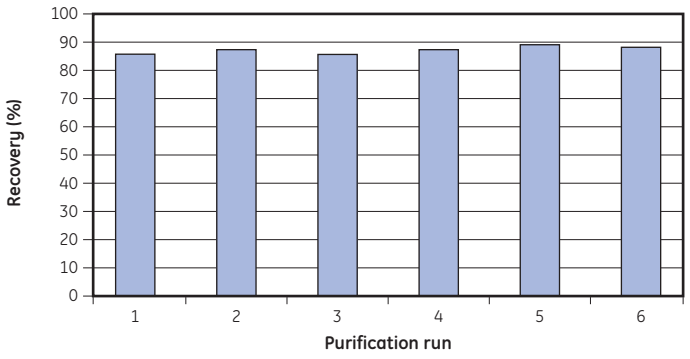
Repeatable antibody purification with high purity

To show the efficiency and repeatability of Protein A Mag Sepharose Xtra and Protein G Mag Sepharose Xtra, six replicate purification runs were performed (Table 4). The load was half of the total binding capacity for each medium. The antibody yield was consistently high (> 80%), and the purity analyzed by SDS-PAGE was > 90%. The data (Figures 5 and 6) shows that the purification runs on Protein A Mag Sepharose Xtra and Protein G Mag Sepharose Xtra were highly repeatable with a relative standard deviation (RSD) of < 2%.

Table 4. Experimental conditions for Protein A Mag Sepharose Xtra and Protein G Mag Sepharose Xtra

Sample	Human IgG spiked in *E. coli* lysate
Sample volume	300 µl
Binding/wash buffer	PBS (140 mM NaCl, 2.7 mM KCl, 10 mM phosphate, pH 7.4)
Elution buffer	100 mM glycine, pH 2.8

A). Protein A Mag Sepharose Xtra



B). Protein G Mag Sepharose Xtra

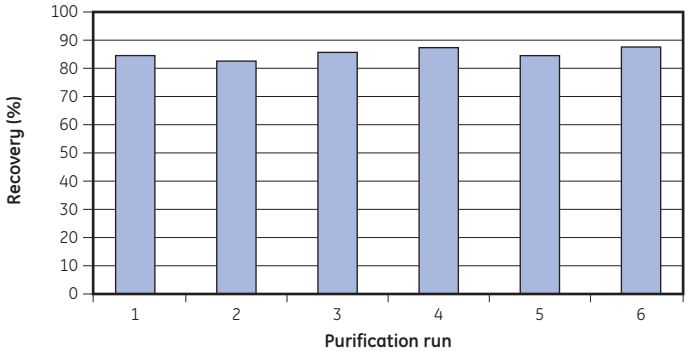


Fig 5. The recovery for six replicate purification runs was consistently > 80% (RSD < 2%).

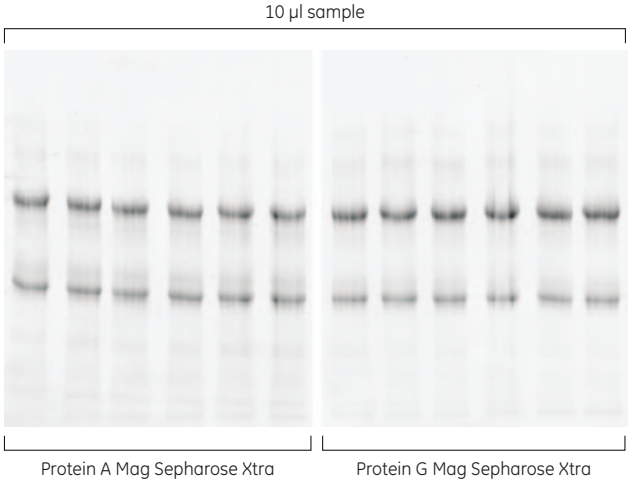


Fig 6. SDS-PAGE stained with Deep Purple™ Total Protein Stain under reducing conditions. The purity obtained for six replicate runs was above 90% (RSD < 2%).

Expression screening study

We performed a comparative study using magnetic beads and a chromatographic column to determine the expression of human IgG_1 produced in CHO cells (Table 5). Samples containing IgG_1 were continuously taken out from a bioreactor during a two-week cell culture period. The samples were analyzed using Protein A Mag Sepharose Xtra beads and a 1 ml HiTrap MabSelect SuRe™ column and the data shows that the two techniques produced similar results (Fig 7). However, the use of Protein A Mag Sepharose Xtra had the following advantages over the 1 ml HiTrap MabSelect™ SuRe column: (i) Simplified handling of samples without the need for a chromatographic system, (ii) Ability to analyze several samples simultaneously thus reducing the total time required for analysis.

Table 5. Experimental conditions for the expression screening study

	Protein A Mag Sepharose Xtra	HiTrap MAbSelect SuRe
Medium volume	10 µl	1 ml
Binding/wash buffer	PBS (140 mM NaCl, 2.7 mM KCl, 10 mM phosphate, pH 7.4)	PBS (140 mM NaCl, 2.7 mM KCl, 10 mM phosphate, pH 7.4), 0.05% Tween 20
Elution buffer	100 mM glycine, pH 2.8	100 mM sodium phosphate, pH 3.0

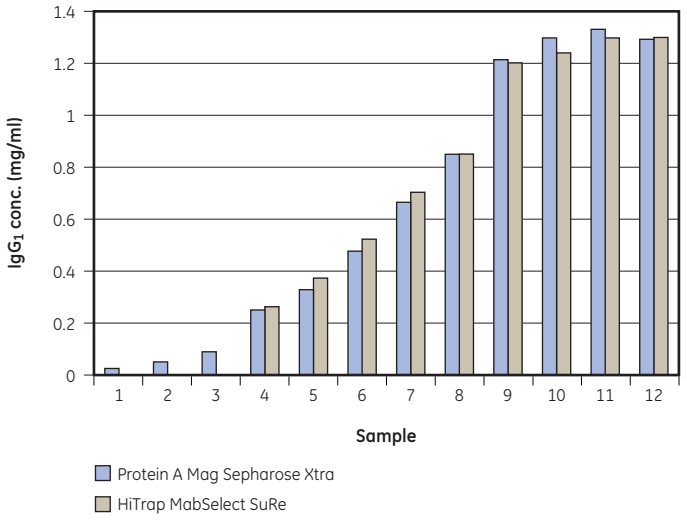


Fig 7. Human IgG_1 concentrations from a comparative performance evaluation of Protein A Mag Sepharose Xtra and HiTrap MabSelect SuRe. Note: Samples 1 to 3 were not analyzed using HiTrap MabSelect SuRe.

Purification of large volumes of low expression mouse IgG_{2b}

One of the key advantages of magnetic bead purification is the ability to use different volumes of sample and medium slurry. In this study, low expressed monoclonal mouse IgG_{2b} in 50 ml diluted cell supernatant (0.07 mg Ab/ml) was successfully purified and concentrated using 1.75 ml Protein A Mag Sepharose Xtra. The sample load was 3.5 mg and the experiment was performed in duplicate. The results show high specificity according to SDS-PAGE analysis (Fig 8) and recoveries of ~ 70%. The purified mouse IgG_{2b} was concentrated from 50 ml to 3.5 ml.

Table 6. Experimental conditions for Protein G Mag Sepharose Xtra

Sample	Mouse IgG_{2b} from hybridoma cells
Sample volume	50 ml (25 ml cell supernatant diluted with 25 ml binding buffer)
Medium slurry volume	1.75 ml
Binding/wash buffer	PBS (140 mM NaCl, 2.7 mM KCl, 10 mM phosphate, pH 7.4)
Elution buffer	100 mM glycine, pH 2.8

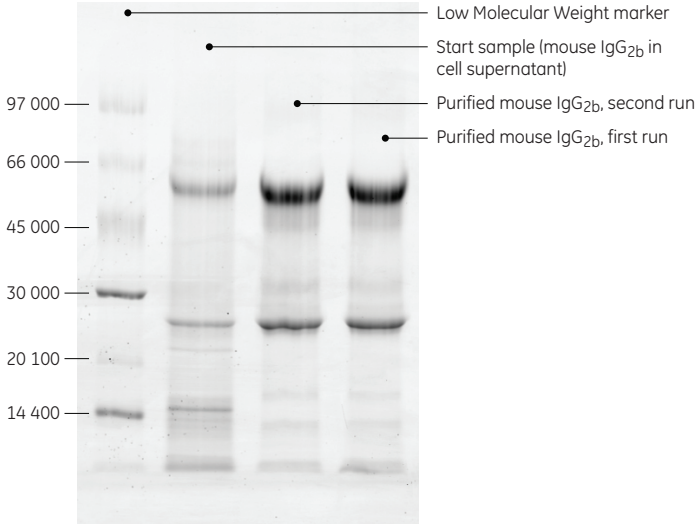


Fig 8. SDS-PAGE stained with Deep Purple Total Protein Stain under reducing conditions. The purification of 50 ml of low expression mouse IgG_{2b} in a cell supernatant resulted in high purity (> 90%).

Two different protein A and protein G Mag Sepharose product lines

Protein A Mag Sepharose and Protein G Mag Sepharose are optimized for efficient enrichment of low abundant proteins by immunoprecipitation techniques. Protein A Mag Sepharose Xtra and Protein G Mag Sepharose Xtra are optimized for small-scale purification/screening of antibodies. For more information about Protein A Mag Sepharose and Protein G Mag Sepharose see Data file: Protein A Mag Sepharose, Protein G Mag Sepharose, NHS Mag Sepharose, GE Healthcare, 28-9539-39, Edition AA (2009).

Ordering Information

Products	Quantity	Code No.
Protein A Mag Sepharose Xtra	2 × 1 ml 10% medium slurry	28-9670-56
Protein A Mag Sepharose Xtra	5 × 1 ml 10% medium slurry	28-9670-62
Protein G Mag Sepharose Xtra	2 × 1 ml 10% medium slurry	28-9670-66
Protein G Mag Sepharose Xtra	5 × 1 ml 10% medium slurry	28-9670-70

Related products	Quantity	Code No.
MagRack 6	1	28-9489-64
Ab Buffer Kit	1	28-9030-59
HiTrap Desalting	5 × 5 ml	17-1408-01
PD MiniTrap G-25	50 columns	28-9180-07
His Mag Sepharose Ni	2 × 1 ml 5% medium slurry	28-9673-88
His Mag Sepharose Ni	5 × 1 ml 5% medium slurry	28-9673-90
His Mag Sepharose Ni	10 × 1 ml 5% medium slurry	28-9799-17
Protein A Mag Sepharose	1 × 500 µl 20% medium slurry	28-9440-06
Protein A Mag Sepharose	4 × 500 µl 20% medium slurry	28-9513-78
Protein G Mag Sepharose	1 × 500 µl 20% medium slurry	28-9440-08
Protein G Mag Sepharose	4 × 500 µl 20% medium slurry	28-9513-79
NHS Mag Sepharose	1 × 500 µl 20% medium slurry	28-9440-09
NHS Mag Sepharose	4 × 500 µl 20% medium slurry	28-9513-80
TiO_2 Mag Sepharose	1 × 500 µl 20% medium slurry	28-9440-10
TiO_2 Mag Sepharose	4 × 500 µl 20% medium slurry	28-9513-77

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28-9768-01 AA 06/2010