



DATA SHEET

MAGicBeads Protein G



- Super-paramagnetic porous beads
- High binding capacity >65 mg IgG/mL
- High affinity to IgG species from multiple sources
- Minimal non-specific interaction

MAGicBeads Protein G

MAGic™Beads Protein G provides a simple and effective system for isolation of antibodies using magnetic separation techniques. This product is intended for purification of monoclonal antibodies from cell culture harvests, such as from hybridoma or CHO cells.

The beads are spherical porous agarose beads (average size of 55 µm) with an optimal pore size for antibodies, that have been magnetized to become super-paramagnetic. Agarose-based beads are inert and hydrophilic, preventing nonspecific interactions and are compatible with all kinds of biomolecules. A recombinant Protein G has subsequently been coupled to this bead to produce MAGicBeads Protein G. See Table 1 for the bead characteristics.

The black beads are easily observed by the naked eye, making them easy to follow and collect, and they do not aggregate, which facilitates the resuspension. The quantity of the high-capacity beads can easily be scaled up or down to match the antibody concentration and sample volume.

Table 1. Main characteristics of MAGicBeads Protein G

Product	MAGicBeads Protein G, 10% bead suspension
Target molecule	Antibodies (IgG), bound via the F _c -region
Matrix	Super-paramagnetic porous agarose
Ligand	Recombinant Protein G
Average particle size (DV50) ¹	55 µm
Maximum binding capacity ^{2,3}	>65 mg IgG/mL settled beads
Practical binding capacity ^{2,4}	45 mg IgG/mL settled beads
Chemical stability	Compatible with most standard aqueous buffers used for protein purification and 20% ethanol. Not compatible with citrate buffer and NaOH. Should not be stored at low pH for prolonged time
Storage	+2 to +8°C in 20% ethanol.

¹ The median particle size of the cumulative volume distribution.

² Binding capacity for human polyclonal IgG at a titer of 5 mg IgG/mL.

³ The maximum binding capacity is performed under IgG excess to allow all ligands to bind to targets (saturated conditions).

⁴ The practical binding capacity is at 90% binding of targets to ligands. This value is always lower than the maximum capacity.

Applications

Principle

MAGicBeads Protein G is a magnetic affinity separation media with high affinity for antibodies, specifically IgG, from various species. This capture step generates high yields and purities of antibodies in a single step and is therefore commonly used in antibody purification processes or in immunoprecipitation protocols. The 55 µm super-paramagnetic MAGicBeads are based on porous agarose.

Varied affinities for IgGs from different sources

Protein G binds IgG antibodies with high affinity across a broad range of species and subclasses (see Table 2 below), making it more versatile than Protein A in many applications. Its broad binding profile makes Protein G a valuable tool for IgG isolation and purification, particularly in small-scale purification workflows and immunoprecipitation assays.

Table 2. Antibody affinity to Protein A and Protein G

Species	IgG class	Protein A	Protein G	Species	IgG class	Protein A	Protein G
Human	IgG1	++	++	Rabbit	IgG	++	++
	IgG2	++	++	Hamster	IgG	+	++
	IgG3	–	++	Guinea Pig	IgG	++	+
	IgG4	+	++	Bovine	IgG	+	++
	IgA	+	–	Horse	IgG	+	++
	IgD	+	–	Sheep	IgG	+/-	++
	IgE	+	–	Goat	IgG	+/-	++
	IgM	+	–	Pig	IgG	++	++
Mouse	IgG1	+	++	Chicken	IgY	–	+/-
	IgG2a	++	++	Antibody fragments			
	IgG2b	++	++	Human	Fab	+	+
	IgG2c	+	++		F(ab') ₂	+	+
Rat	IgM	+/-	–		scFv	+	–
	IgG	++	++		Fc	+	+
	IgG1	+/-	+		κ	–	–
	IgG2a	+/-	++		λ	–	–
	IgG2b	+/-	+				
	IgG2c	+/-	+				
	IgM	+/-	–				
++ strong		+ moderate	– low	+/- needs evaluation			

References:

Richman DD, Cleveland PH, Oxman MN, and Johnson KM. (1982) The binding of *Staphylococci* Protein A by the sera of different animal species. *J Immunol* **128**, 2300-2305.

Frank MB. (1997) Antibody Binding to Protein A and Protein G beads. In: Frank MB, ed. *Molecular Biology Protocols*. Oklahoma City.

High binding capacity for different sources at varied titers (BC)

There is a positive correlation between binding capacity and antibody concentration. MAGicBeads Protein G has a broad dynamic range in capacity to bind antibodies at decreasing titers (from large scale purifications to screening and low titer detection applications).

For high IgG concentrations ≥ 5 mg/mL:

- Maximum BC: 65-80 mg/mL (human and rabbit IgG)

For low IgG concentrations of 0.1 mg/mL:

- Maximum BC: 18-20 mg/mL (human IgG)
- Maximum BC: 13 mg/mL (mouse IgG)

Similar binding capacity values are obtained regardless of the purity of the IgG loaded (e.g., pre-purified IgG vs. IgG expressed in feed).

To investigate the correlation between antibody titer and binding capacity, a binding study was performed using purified human IgG at concentrations ranging from 0.1 to 5 mg/mL. Beads were added to the antibody samples at optimized bead-to-antibody ratios in PBS buffer and incubated for 1 hour at room temperature under continuous mixing. The experimental conditions are summarized in Table 3. Maximum binding capacities were determined as a function of IgG concentration, and the results are presented in Figure 1.

Table 3. Experimental conditions for binding studies to investigate the correlation between IgG titer and binding capacities. Last column displays the binding capacities obtained under the specified conditions.

#	IgG concentration (mg/mL)	Reaction volume (mL)	Bead volume (μL)	Binding capacities (mg IgG/mL)
1	5	1	50	72
2	2	1	50	32
3	0.1	2	10	19

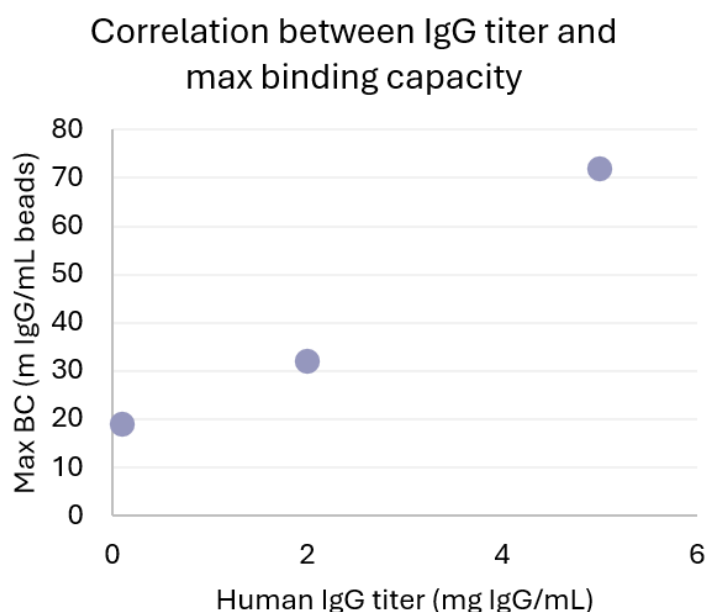


Figure 1. Maximum binding capacities of human IgG (pure) to MAGicBeads Protein G at increasing titers ranging from 0.1 mg/mL up to 5 mg/mL.

Practical binding capacity - calculations

The difference between maximum binding capacity ($Q_{\max}$) and the practical binding capacity is that the former test is performed under an IgG excess at a defined titer to allow all ligands to bind to the target, whereas the practical binding capacity targets a 90% binding of targets to ligands. The measured $Q_{\max}$ is therefore always higher than the practical binding capacity.

The practical binding capacity at different antibody titers should be tested for each specific antibody to obtain correct capacity values. Based on the practical binding capacity values at different titers, the optimal amount of target-bead ratio can be calculated. See the instructions for these calculations.

High yields achieved in purifications from complex feeds

In monoclonal antibody purifications, the yield is of utmost importance and should preferably exceed 80%. To verify the yield obtained using MAGicBeads Protein G, purifications were conducted on multiple batches and evaluated.

IgG1 expressed in Chinese Hamster Ovary (CHO) cells at a titer of 1.3 mg/mL was purified on MAGicBeads Protein G. 464 μ L 10% bead slurry was equilibrated in PBS and 1 mL of antibody feed was added. The suspension was incubated for an hour under stirring and, after 3 wash steps, was eluted in 100 mM glycine buffer, pH 2.7. For recoveries from purifications on different MAGicBeads Protein G batches, see Table 4 below.

Table 4. IgG1 elution recoveries from MAGicBeads Protein G after 1-hour adsorption. IgG1 was expressed in CHO cells at a titer of 1.3 mg/mL.

MAGicBeads Protein G batch	Eluted IgG1 recovery (%)
Batch A	85
Batch B	85
Batch C	82
Batch D	84

To achieve high IgG recoveries exceeding 80%, two consecutive elution steps is recommended. Table 5 and Figure 2 summarizes the amount of IgG recovered in each elution fraction, demonstrating that the majority of IgG was recovered during the first elution. At an IgG expression level of 1.3 mg/mL (Batch C), approximately 63% of the total IgG was recovered in the first elution fraction, while pooling the two elution fractions increased the overall yield to more than 80%. However, the proportion of IgG recovered in each elution step may vary depending on the antibody and the running conditions, as shown in Figure 3.

Table 5. Purification results from an IgG-containing cell culture (Batch C) obtained with a magnetic bead extraction system. First row in each category displays results in 1st elution fraction. Second row, 2nd elution fraction. Third row, all elution fractions pooled.

Feed: 1 mL of 1.30 mg IgG/mL	
Recovered IgG (mg)	0.82 (1 st elution) 0.25 (2 nd elution) 1.07 (Total)
Yield (%)	63 (1 st elution) 19 (2 nd elution) 82 (Total)

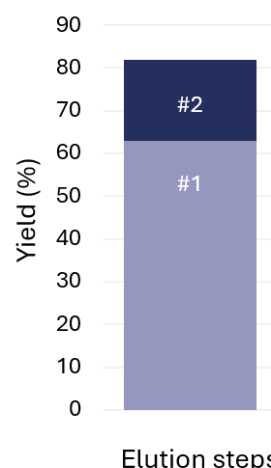


Figure 2. IgG recovery ratios in the two elution fractions.

Low titer feeds

Protein G is commonly used for antibody detection and purification in low-titer expression systems, making high sensitivity and a broad dynamic range essential for reliable performance. To evaluate the performance of MAGicBeads Protein G under these conditions, low-titer mouse IgG feeds were used as samples. Mouse IgG1 and IgG2b were expressed in CHO cells at titers ranging from 50 to 170 µg/mL. For each purification, 50 µL (for titers < 100 µg/mL) or 100 µL (for titers > 100 µg/mL) of 10% MAGicBeads Protein G slurry was equilibrated in PBS before adding 1 mL of antibody feed. The suspension was incubated for 1 hour under stirring, followed by three wash steps. Bound antibodies were subsequently eluted using 100 mM glycine buffer at pH 2.7 in two consecutive 250 µL elution steps. The resulting purification yields are presented in Figure 3, demonstrating the performance of MAGicBeads Protein G across a range of low antibody titers.

To estimate the purity of the antibody eluates, the 260/280 ratios were measured. A 260/280 ratio below 0.8 is generally considered an indication of relatively high purity in antibody eluates, with lower ratios indicating greater purity. The average 260/280 ratio across the elution fractions was 0.62, indicating a high degree of purity.

The average elution yield from the IgG1 purifications was 87% and the elution yield from the IgG2b purification was 82%.

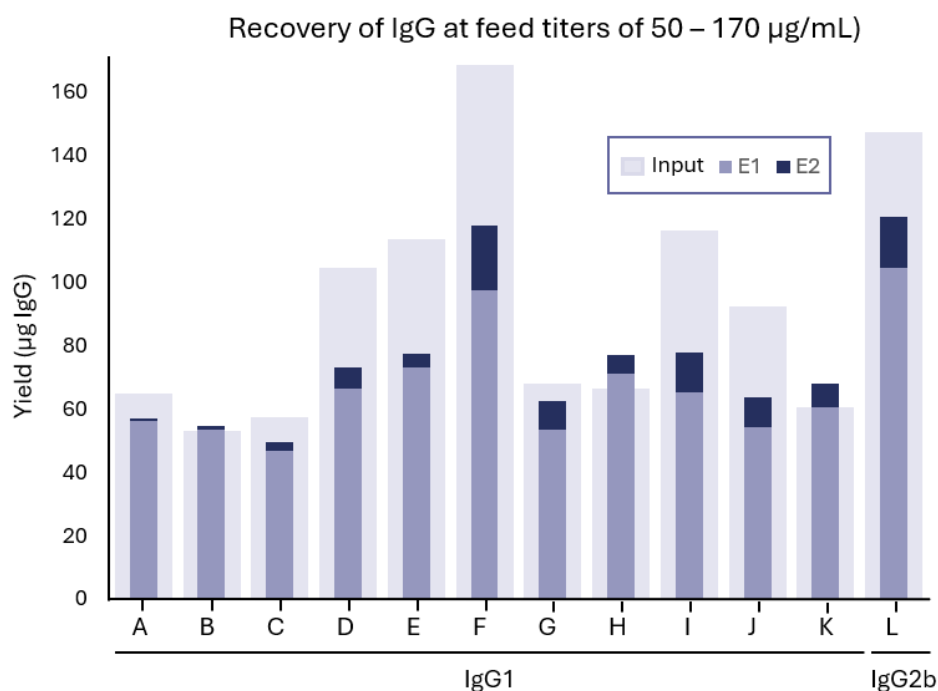


Figure 3. Elution yield from low-titer mouse IgG1 and IgG2b feeds. Elution yields are shown for the first elution step (E1; purple) and second elution step (E2; dark blue), together with the corresponding IgG input amounts (light purple) for purifications performed using MAGicBeads Protein G. A total of 11 IgG1 feeds and 1 IgG2b feed were evaluated.

Yields dependent on pH

The interaction between antibodies and Protein G is destabilized at low pH, and an elution buffer at approximately pH 3 is therefore commonly used for antibody recovery. However, antibodies are prone to aggregation under acidic conditions, making it desirable to perform elution at the highest possible pH while maintaining acceptable recovery. The optimal elution pH is antibody-dependent and must therefore be determined empirically. As elution efficiency generally decreases with increasing pH, a balance must be achieved between maximizing recovery and minimizing aggregation.

To investigate the effect of elution pH, mouse IgG was eluted using 100 mM glycine buffers with pH values ranging from 2.5 to 3.0. Two mL of IgG expressed in *Escherichia coli* cells at a titer of 0.1 mg/mL were purified on 15 µL MAGicBeads Protein G beads. As expected, the elution efficiency was highest at pH 2.5, resulting in an approximately 25% higher recovery compared with pH 3.0 (Figure 4). Depending on the intended application, the trade-off between higher recovery at lower pH and the increased risk of antibody aggregation should be carefully considered.

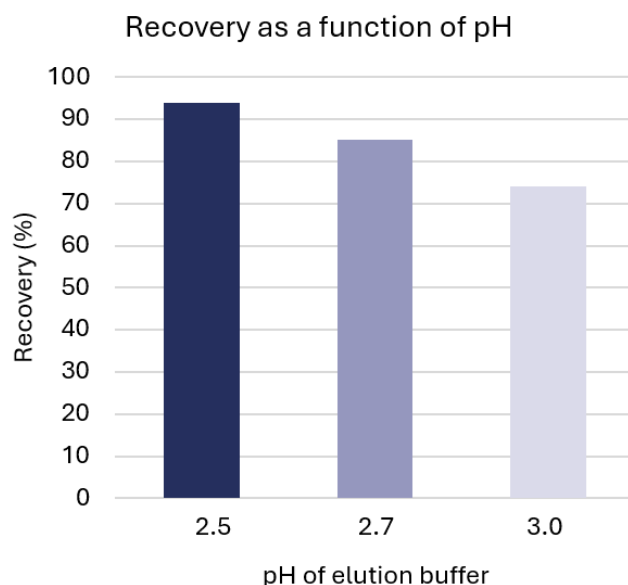


Figure 4. Recovery as a function of elution pH. Recoveries from a purification of a low titer (IgG expression of 0.1 mg/mL) mouse IgG *E. coli* feed using MAGicBeads Protein G are shown following elution with 100 mM glycine buffer at pH 2.5, 2.7, and 3.0.

Super-paramagnetism

MAGicBeads are super-paramagnetic beads, where magnetic separation occurs within seconds at small scale (1 mL), due to our novel proprietary production method. These beads are easily resuspendable without forming any aggregates facilitating the separation process. This is due to the property of the agarose beads being hydrophilic and the lack of a magnetic memory. These properties speed up the protocols at all scales.

Scale-up

The beads are suitable for separations using appropriate magnetic separators, such as the MAGicAccio LAB, MAGicAccio PILOT 50, MAGicAccio PILOT 500, MAGicAccio PILOT 2000 and MAGicAccio PROCESS.

These separators vary from lab scale to medium scale/process development up to large scale/bioprocessing scale.

MAGic BioProcessing's proprietary PROCESS technology simplifies antibody recovery by combining clarification and capture into one scalable process. Using MAGicBeads Protein G to isolate antibodies directly from complex feedstocks, and paired with our automated magnetic separation system, the platform creates a smooth link between upstream and downstream operations, improving efficiency and saving time.

Storage

The MAGicBeads Protein G should be stored as a 10% bead suspension at +2 to +8°C in 20% ethanol.

Ordering information

Products	Quantity	Product No.
MAGicBeads Protein G	1 mL	1400
MAGicBeads Protein G	10 mL	1401
MAGicBeads Protein G	50 mL	1405
MAGicBeads Protein G	100 mL	1410
MAGicBeads Protein G	250 mL	1425
MAGicBeads Protein G	500 mL	1450

Related products	Product No.
MAGicBeads ACT	13XX
MAGicAccio LAB rack	2000
MAGicAccio LAB cube	2100
MAGicAccio PILOT 50	2200
MAGicAccio PILOT 500	2300
MAGicAccio PILOT 2000	2400
MAGicAccio PROCESS	3100

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