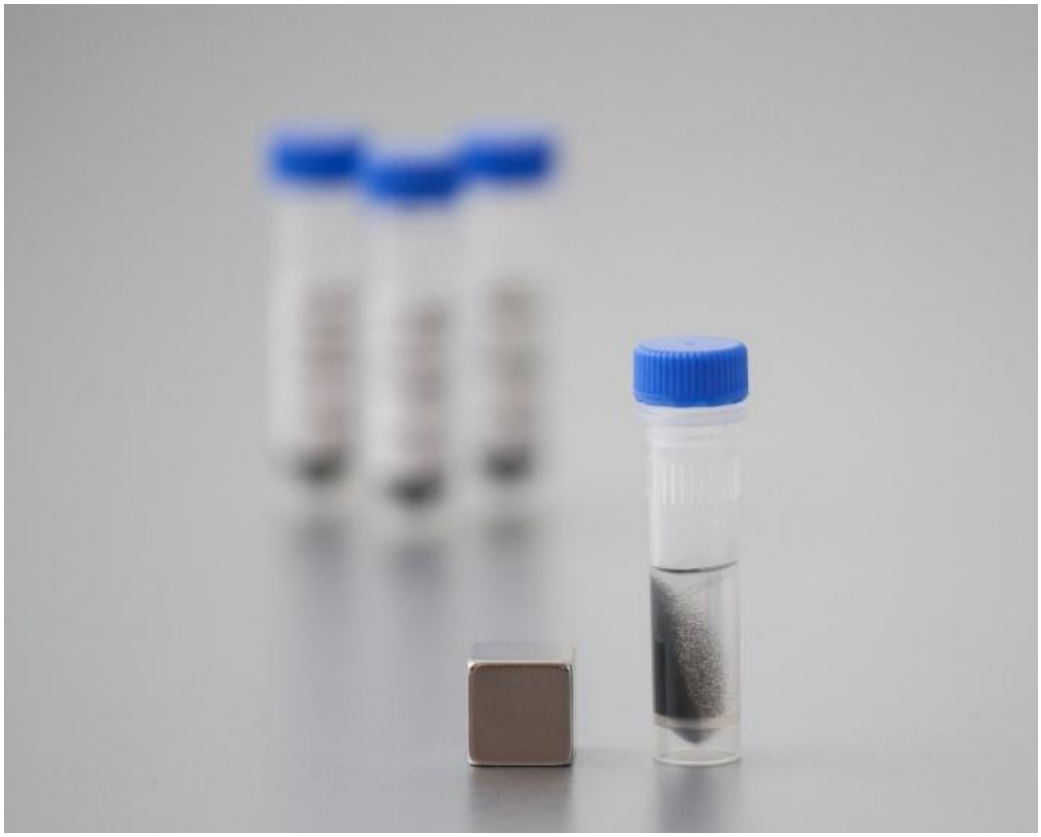
MAGic™
BioProcessing

INSTRUCTION

MAGicBeads Protein G



- Super-paramagnetic porous beads
- Exceptional magnetization and zero magnetic remanence
- High binding capacity >65 mg IgG/mL
- Minimal non-specific interaction

Safety

Please read through this instruction and the associated Safety Data Sheet (SDS) carefully before using MAGic™Beads Protein G.

Intended use

This product is intended for purification of antibodies from cell culture broth and ascites fluid, as well as total IgG from serum.

For research use only.

Bead suspension

The product is a 10% bead suspension; however, it may appear to contain a larger bead volume when bead–bead interactions occur, altering bead configurations. This phenomenon is batch-specific but does not affect bead performance.

General information

MAGicBeads Protein G provides a simple and effective system for small to medium scale isolation of antibodies using magnetic separation techniques. The quantity of high-capacity beads can easily be scaled up or down to match the antibody concentration and sample volume. The beads are suitable for separations using appropriate magnetic separators, such as the MAGicAccio line.

MAGicBeads Protein G consists of super-paramagnetic agarose beads covalently coupled with a recombinant Protein G ligand. This product is intended for purification of antibodies from cell culture harvests, such as from hybridoma or CHO cells. Protein G binds to primarily IgG. It binds to a broader range of antibody isotypes among mouse and human IgG subclasses compared to Protein A.

The MAGicBeads Protein G magnetic beads are easily attracted to external magnets, allowing efficient separation. The agarose matrix enables minimal nonspecific binding of proteins due to its hydrophilic nature. 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 capacity is highly dependent on the nature of the antibody and the antibody concentration in the sample. MAGicBeads Protein G can be used multiple times.

Product data

Table 1. Characteristics for MAGicBeads Protein G

Product	MAGicBeads Protein G, 10% bead suspension
Target molecule	Antibodies (IgG)
Matrix	Super-paramagnetic porous agarose
Ligand	Native recombinant Protein G
Average particle size (DV50)[1]	~55 µm
Maximum binding capacity[2]	> 65 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. 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.

Materials supplied

- MAGicBeads Protein G supplied as a 10% bead suspension in 20% ethanol. 10 mL 10% bead suspension contains 1 mL beads.

Additional materials needed

- **Washing buffer** – For washing of beads, use PBS (137 mM NaCl, 2.7 mM KCl, 10 mM phosphate, pH 7.4) or similar recipe, e.g., 15 mM phosphate pH 7.4, 150 mM NaCl.
- **Elution buffer** – For release of antibodies from beads, use 100 mM glycine pH 2.5-3.0, or conditions optimized for a specific antibody. Avoid using citrate due to potential interference with the analytical readout.
- **Neutralization buffer** – To neutralize eluted antibodies, use 2 M Tris-HCl, pH 9.0.
- **Storage buffer** – Store beads in 20% ethanol.
- **Mixer** – Mix samples during incubations using an end-over-end mixer, a benchtop shaker, or a rocking table. Manual inversion of the vial can also be applied.
- **Magnetic separator** – MAGicAccio LAB (Product No. 2000, 2100) are suitable for separations in 0.5–5 mL volumes. For separation of volumes larger than 5 mL, use MAGicAccio PILOT 50 (Product No. 2200) for volumes up to 50 mL, MAGicAccio PILOT 500 (Product No. 2300) for volumes up to 500 mL, or MAGicAccio PILOT 2000 (Product No. 2400) for volumes up to 2000 mL.
- Additional vials/tubes, pipettes and pipette tips.

Handling instructions

Dispensing the bead suspension

- The bead suspension should be well suspended before dispensing. Mix thoroughly by manual inversion, between each pipetting from the vial.

Magnetic bead separation

- MAGicAccio LAB can be used to collect the beads from liquid volumes up to 5 mL. For volumes from 5 mL up to 50 mL it is recommended to use the MAGicAccio PILOT 50 separator. Use the MAGicAccio PILOT 500 separator for volumes up to 500 mL or the MAGicAccio PILOT 2000 for volume up to 2000 mL. MAGicAccio PROCESS is an integrated separator system made for larger bioprocess volumes. Refer to the manual of the separators for detailed instructions.
- Use the magnetic separator to attract the magnetic agarose beads to the wall of the test tube or bottle before each liquid removal step.
- Remove liquid carefully, trying not to disturb the magnetic beads. To avoid sample loss, make sure that no beads are removed.
- Move the tube away from the magnetic field, add new liquid and resuspend the beads by mixing.

Incubation

- Incubations should be performed with continuous mixing, using either an end-over-end apparatus, a bench-top shaker, or a rocking table. Short incubations, e.g., for elution, can be performed by using manual mixing/inversion of the test tube or bottle.
- Binding and elution can be performed at room temperature, as well as in a cold room.
- Generally, when purifying antibodies of low concentrations the amount of beads should be increased.

Important operation parameters

Bead input

- The amount of beads and the binding time strongly depends on the antibody concentration in the sample.

Binding

- Purification can be performed directly in cell culture media, without diluting the sample. However, the pH must be within the given range. Always check the pH of the sample and, if necessary, adjust accordingly by using a suitable high molar Tris-buffer.
- Glycerol applied to the samples might reduce non-specific binding.
- If the binding capacity is too low, reduce pH in washing buffer until optimized conditions are obtained.

Washing

- In most applications, it is sufficient to wash the beads with PBS (137 mM NaCl, 2.7 mM KCl, 10 mM phosphate, pH 7.4).
- In some cases, a more stringent wash using high salt, e.g., 0.5–1 M NaCl, or the addition of a suitable detergent, e.g., 0.1–1.0% Tween® 20, can be beneficial. A wash with a minor pH decrease, that do not elute the target protein, can also remove weakly adsorbed impurities.

Elution

- The recommended elution buffer is 100 mM glycine, pH 2.5-3.0, for many antibodies. Avoid citrate buffers due to potential interference with the analytical readout.
- The adsorbed antibodies are generally eluted within 2 min of mixing with elution buffer.
- If low yields are obtained, increase incubation time and/ or decrease pH in elution buffer.
- For neutralization of eluted antibodies, add, e.g., 1/10 fraction volume of 2 M Tris-HCl, pH 9.0, to each elution fraction. It is recommended to conduct the neutralization step promptly after elution to prevent potential antibody aggregation.
- Balance the number of elutions with regards to total yield and possibly concentration issues in the final total elution volume. Pooling elution fractions gives a higher yield in a larger volume, but at a lower concentration.

- If needed, concentrate the neutralized and/or desalted antibody using a suitable technique.
- Always analyze the elution efficiency by estimating the ratio between eluted and adsorbed antibody and perform a functional characterization of the eluted and desalted antibody.

Note: Bead volume is the volume of settled beads, i.e., 10% of the delivered bead suspension volume. 10 mL bead suspension corresponds to 1 mL bead volume.

Optimizing elution

- The elution buffer may need optimization, as different immunoglobulins elute at different pH values depending on species and subclass. Some immunoglobulins are also more sensitive (acid-labile) towards low pH. Recommended elution buffer is as stated 100 mM glycine, pH 2.5-3.0, but other elution buffers may also be evaluated (avoid citrate). Since antibodies tend to bind with a high affinity to Protein G it is common that pH in elution buffer needs to be reduced to achieve optimized conditions. This can additionally be combined with longer elution incubations.
- If the purified antibody tends to precipitate, the pH of the solution might be close to the pI of the antibody; a common cause of precipitation for many proteins. If working with a recombinant antibody, the pI can be calculated (look for online resources, e.g., http://web.expasy.org/protparam/), since the primary amino acid residue sequence can be derived from the corresponding cDNA sequence. Change to a buffer with a pH at least 0.5–1.0 units away from the pI. When the pI is unknown, such as from antibodies produced in hybridoma, optimize by testing different buffer pH.

Regeneration of magnetic beads

- The beads can normally be used multiple times without loss in binding capacity and selectivity.
- To regenerate the beads, wash a minimum of three times with 10 bead volumes elution buffer and twice with 10 bead volumes PBS.
- When reusing beads, it is recommended to use the beads for purification of the same antibody to avoid potential cross-contamination between different antibodies.

Storage

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

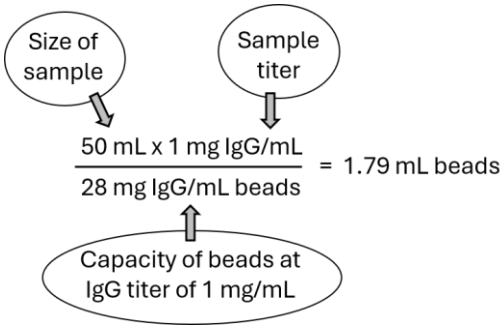
Optimization

The general recommendations in this manual are suitable for most antibodies and sample types. However, optimization may be needed to obtain maximum recovery. Parameters that may require optimization are:

- Binding time
- Amount of beads
- Buffers (washing and elution buffer)
- Number of washes
- Elution time

Antibody binding capacity

- The binding kinetics between Protein G-beads and antibody depends on the concentration (titer) of antibodies in the sample.
- Higher antibody concentrations generally require more beads to achieve the same percentage of adsorption within a given time.
- The practical binding capacity (BC) for MAGicBeads Protein G at different concentrations of IgG antibodies, as set to 90% adsorption for 1 mL beads and 1 hour incubation should be tested for your specific antibody to obtain most optimal ratios/capacities.
- If no experimental data are available for your antibody, Table 2 provides estimated practical binding capacities for samples with low, medium, and high antibody titers. These values can be used as a starting point for estimating the required bead volume.
- Following provided data in table 2, in a sample of 50 mL CHO-cell broth with an antibody titer of 1 mg IgG/mL, the amount of beads to use to obtain 70-80% adsorption after 2 hours, would be 1.79 mL beads (see below).



- To obtain higher than 70-80% adsorption, either more beads should be used or an extended incubation time. This applies especially to low titers.

Table 2: Practical binding capacity guidelines at different concentrations of human IgG antibody per mL of Protein G magnetic beads at 1 hour adsorption.

Human IgG (mg/mL sample)	Binding capacity (mg IgG/mL beads)
0.1	10
1	28
5	45

Antibody purification protocol

Intended use

The product is intended for purification of antibodies from cell culture media at lab scale. If using the MAGicAccio PILOT 50, MAGicSep PILOT 500, MAGicAccio PILOT 2000 and/or MAGicAccio PROCESS separators, please read the product manuals for those for further instructions.

Bead pre-treatment

1. Calculate the amount of beads to use for your specific purification (see section above).
2. Mix bead suspension thoroughly.
3. Dispense the required amount of 10% bead suspension in a suitable tube or bottle.
4. Remove liquid by magnetic separation.
5. Resuspend beads in 10 bead volumes PBS.
6. Remove the liquid.

Sample application

7. Adjust pH of sample if necessary. pH should be in the range 5–7.4.
8. Resuspend beads with a small amount sample media and transfer to the main sample container.
9. Incubate with continuous mixing during the adsorption step.
10. Remove the liquid after the set adsorption time. Save unbound fraction for SDS-PAGE if required.

Washing

11. Add 10 bead volumes binding buffer, resuspend the beads, and mix for 1 min.
12. Remove the liquid.
13. Perform steps 11 and 12 at least three times.

Elution

14. Add 5–10 bead volumes of elution buffer.
15. Resuspend the beads and mix for 2 min.
16. Remove and collect the elution fraction. Generally, a very high fraction of bound antibodies is found in the first elution fraction, when eluting with 10 bead volumes. If beads have been accidently transferred with

the collected elution fraction, a second separation can be performed, and the eluted fraction transferred to another new tube.

17. Repeat elution step if necessary.
18. Regenerate beads and resuspend in storage solution.

Note: 1 mL bead suspension corresponds to 0.1 mL bead volume. 0.1 mL bead volume is the same as 0.1 mL settled beads.

Practical notes

- Beads caught in the lid or on the walls of the reaction vial can be recovered by washing with solution using a pipette or removed with a quick spin in a microcentrifuge.
- If low amount of antibody is recovered, increase the amount of magnetic beads and/or increase the time of incubation.
- It is recommended to optimize the coupling time of antibodies to beads depending on sample source and antibody concentration.
- If foam has been developed during the adsorption step of the cell culture media, it will usually be removed during the subsequent wash step with PBS.
- If the antibody is sensitive to the low pH during elution, optimize elution conditions to identify the highest pH needed for efficient elution. Always neutralize and/or desalt the eluted fraction. Keep exposure of the antibody to extreme pH to a minimum.
- At an unexpected low yield, verify that the IgG isotype indeed binds Protein G, analyze elution efficiency by estimating the ratio between eluted and adsorbed antibody, or elute the beads with 8 M urea and perform an SDS-PAGE to visually confirm possible non-eluting antibodies.
- When reusing beads, it is recommended to use the beads for purification of the same antibody to avoid any cross-contamination between purification runs.
- If there is a need to elute the bound antibody in a smaller volume than is possible with the magnetic separators, the magnetic beads can be transferred and eluted using a basic gravity flow column setup.

Disclaimer

The product is not fully tested. For research use only.

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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