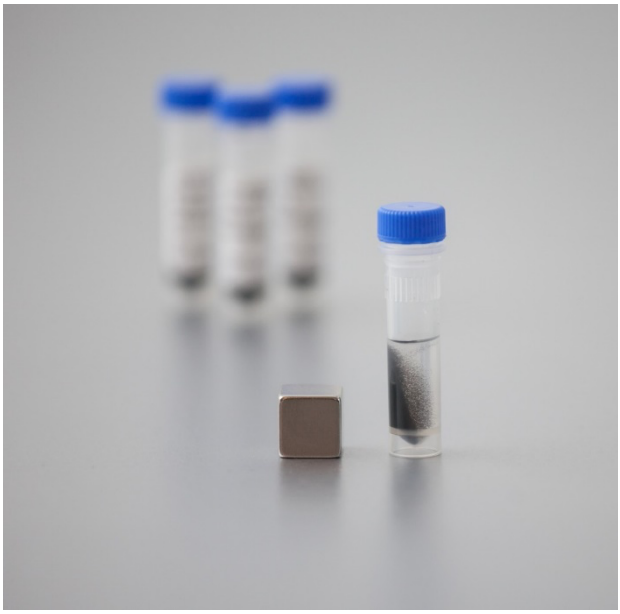
MAGIC BEADS

MAGic™Beads mAb

Alkali-stable protein A magnetic agarose beads

User Instruction



MAGic Bioprocessing AB

Revision date 2020-03

Table of Contents

Please read through this manual carefully before using MAGic™Beads mAb.

Intended use

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

1. General information

MAGic™Beads mAb 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 MAGic™Accio LAB (Product No. 2000, 2100), MAGic™Accio PILOT 50 (Product No. 2200), MAGic™Accio PILOT 500 (Product No. 2300) and MAGic™Accio PILOT 2000 (Product No. 2400)

MAGic™Beads mAb consists of super-paramagnetic agarose beads covalently coupled with an alkali-resistant recombinant protein A ligand. The beads can be sanitized with up to 0.2 M NaOH. This product is intended for purification of monoclonal antibodies from cell culture harvests, such as from hybridoma or CHO cells. For purification of antibodies from serum and ascites, we recommend the extra-high capacity resin MAGic™Beads SerA (Product No. 1200).

The MAGic™Beads mAb magnetic particles 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 antibody concentration in the sample. The practical binding capacity varies from 5 mg IgG/ml at low concentrations (0.05 mg/ml) up to 45 mg IgG/ml at high concentrations (6 mg/ml). The maximum binding capacity is 65 mg of human IgG per ml beads. MAGic™Beads mAb can be used multiple times without noticeable loss of binding selectivity and capacity.

2. Antibody affinity to protein A and protein G

Table 1. Binding strengths

		Protein A	Protein G			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	**++**	**++**				
	IgG2b	**++**	**++**		**Antibody fragments**		
	IgG2c	**+**	**++**	**Human**	**Fab**	**+**	**+**
	IgM	**+/–**	**–**		**F(ab')2**	**+**	**+**
Rat	**IgG**	**++**	**++**		**scFv**	**+**	**–**
	IgG1	**+/–**	**+**		**Fc**	**+**	**+**
	IgG2a	**+/–**	**++**		**κ**	**–**	**–**
	IgG2b	**+/–**	**+**		**λ**	**–**	**–**
	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.

3. Product data

Table 2. Characteristics for MAGic™Beads Alkali A

Matrix	Super-paramagnetic agarose
Product	MAGic™Beads mAb, 10% bead suspension
Ligand	Alkali-resistant recombinant protein A
Particle size	45–165 µm
Practical binding capacity[1]	45 mg IgG/ml settled beads
Maximum binding capacity	>65 mg IgG/ml
Binding conditions	Directly in cell culture media, pH 6–8
Elution conditions[2]	100 mM citrate pH 2.8 100 mM glycine, pH 2.8
Alkali sanitization	0.1 M NaOH for 16 hours (90% retained activity) 0.2 M NaOH for 8 hours (90% retained activity)
Storage	+2 to +8°C in PBS with 20% ethanol.
Stability[3]	18 months
Protein A ligand leakage[4]	10–100 ng/mg IgG (10–100 ppm)
Reusability	Can be reused multiple times

1 Binding capacity at above 6 mg IgG/ml.
2 Some antibodies may require different elution conditions.
3 Data of product stability is continuously updated based on ongoing stability studies.
4 Protein A ligand leakage in the acidic elution fraction with 1 minute contact time at room temperature was determined using a Protein A ELISA kit (#03-96) from Immun System I.M.S AB, Sweden.

4. Materials supplied

- MAGic™Beads mAb supplied as a 10% bead suspension in PBS with 20% ethanol. 10 ml 10% bead suspension contains 1 ml beads.

5. 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 citrate, pH 2.8, 100 mM glycine, pH 2.8, or conditions optimized for a specific antibody.
- **Neutralization buffer** – To neutralize eluted antibodies, use 2 M Tris-HCl, pH 9.0.
- **Sanitization buffer** – To sanitize the beads during regeneration, use 0.1–0.2 M NaOH.
- **Storage buffer** – Store beads in PBS containing 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** – MAGic™Accio LAB (Product No. 2000, 2100) are suitable for separations in 0.5–5 ml volumes. For separation of volumes larger than 5 ml, use MAGic™Accio PILOT 50 (Product No. 2200) for volumes up to 50 ml, MAGic™Accio PILOT 500 (Product No. 2300) for volumes up to 500 ml, or MAGic™Accio PILOT 2000 (Product No. 2400) for volumes up to 2000 ml(Section 13).
- Additional vials/tubes, pipettes and pipette tips.

6. Handling instructions

Dispensing the bead suspension

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

Magnetic bead separation

- MAGic™Accio 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 MAGic™Accio PILOT 50 separator. Use the MAGic™Accio PILOT 500 separator for volumes up to 500 ml or the MAGic™Accio PILOT 2000 for volume up to 2000 ml (Section 13). 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 (Section 8).

7. Product operation

Intended use

- This product is intended for purification of antibodies from cell culture media.

Bead input

- The amount of beads and the binding time strongly depends on the antibody concentration in the sample. See Section 8 for advice.

Binding

- The MAGic™Beads Alkali A bind immunoglobulins with various affinity (Table 1), in the range of pH 6–8.
- 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.

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.

Elution

- The recommended elution buffer is 100 mM citrate, pH 2.8, or 100 mM glycine, pH 2.8, for most antibodies.
- The adsorbed antibodies are generally eluted within 1 min of mixing with 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 (Table 3).

Table 3: Final pH after addition of various volumes of 2 M Tris-HCl, pH 9.0 to 60 mM citrate, pH 3.0 (in-house data)

Vol Tris-HCl (ml)	Vol citrate (ml)	Final pH
0.1	1	7.2
0.15	1	8.1
0.2	1	8.4

- Normally, 88% of bound material is found in the first fraction, 10% in the second, and 2% in the third (in-house data for human and rabbit IgG using 10 bead volume elution fractions).
- 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. Optimized elution buffers include, e.g., 60–200 mM citrate with pH 2.6–3.4 or 100 mM glycine with pH 2.8. For high pH options, consult current literature and/or ready-made elution buffers from other commercial sources.
- If the purified monoclonal 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 monoclonal 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 particles for purification of the same antibody to avoid potential cross-contamination between different antibodies.

Cleaning

- In some samples, strongly bound substances are not fully released and washed away by regeneration. Further cleaning can be evaluated, e.g., using 10 bead volumes of 3 M NaCl for 15 min or 10 bead volumes of 6 M urea for 10 min.

Sanitization

- MAGic™Beads mAb retains 90% of its activity after 16 hours of exposure to 0.1 M NaOH. Perform sanitization as needed in cycles of 15 to 30 min.

Storage

- The MAGic™Beads mAb should be stored as a 10% bead suspension at +2 to +8°C in PBS containing 20% ethanol.

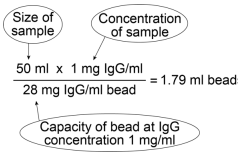
Optimization

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

8. Antibody binding capacity

- The binding kinetics between protein A-beads and antibody depends on the concentration of antibodies in the sample.
- Figure 1 and Table 4, demonstrates the practical binding capacity for MAGic™Beads PrtA at different concentrations of rabbit IgG antibodies, as set to 90% adsorption for 1 ml beads and 1 hour incubation. Use these data as direct guideline for MAGic™Beads mAb.
- Following provided data, 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 >90% adsorption after 2 hours, would be 1.79 ml beads (see below).



- To obtain higher than 90% adsorption, either more beads should be used or an extended incubation time.

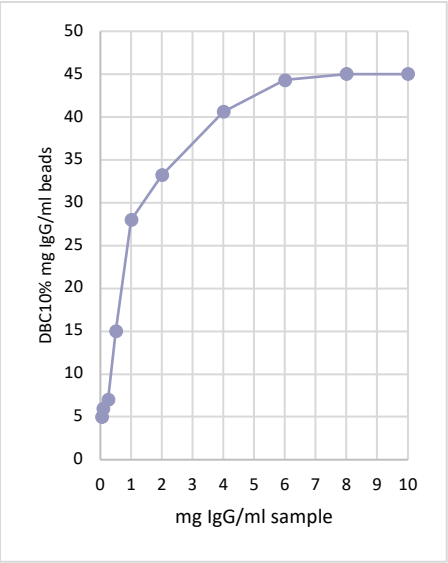


Fig 1. Practical binding capacity for IgG adsorption using magnetic beads, as exemplified with MAGic™Beads PrtA, varies with different concentrations of antibody. Sample points show 90% adsorption at 1 hour. incubation per ml of beads.

Table 4: Practical binding capacity at different concentrations of rabbit IgG antibody per ml of magnetic beads, as exemplified with MAGic™Beads PrtA, at 1 hour adsorption.

Rabbit IgG (mg/ml sample)	Binding capacity (mg IgG/ml beads)
0.05	5
0.1	6
0.25	7
0.5	15
1	28
2	33.2
4	40.6
6	44.3
8	45
10	45

9. Antibody purification protocol

Intended use

The product is intended for purification of antibodies from cell culture media. Beforehand, verify the affinity of each individual antibody to protein A (Table 1).

If using the MAGic™Accio PILOT 50, MAGicSep PILOT 500 and/or MAGic™Accio PILOT 2000 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, using Section 8 as guidance.
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 6–8.
8. Resuspend beads with as mall 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 1 min.
16. Remove and collect the elution fraction. Generally, ~90% of bound antibody 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 yet another new tube.
17. Repeat elution step if necessary.
18. Regenerate beads and resuspend in storage solution (Section 7).

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.

10. 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 A (Table 1), 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.
- A low ppm level leakage of protein A from beads occurs during storage of the product. It is therefore recommended to perform a blank run of the beads before first time use or after long term storage, i.e., several months. The following procedure may be used: Wash with 10 bead volumes of PBS, wash for 15 min with 10 bead volumes of elution buffer, and finally wash with 10 bead volumes of PBS.
- 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.

11. Performance

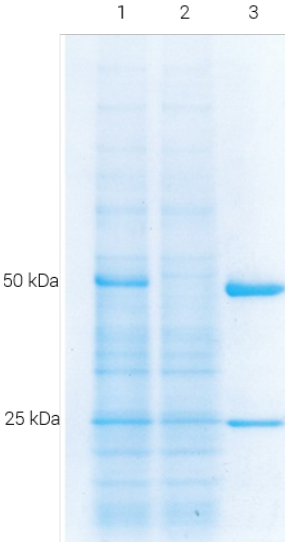


Fig 2. Example of purification at low concentrations. A 20 ml CHO cell culture harvest, with a human IgG titer at 0.020–0.025 mg/ml. The capacity at 5 mg IgG/ml beads, at 0.05 mg IgG/ml, were obtained from Table 4. The amount of beads to use were calculated: (20 ml * 0.025 mg IgG/ml) / (5 mg/ml bead) = 0.1 ml. Incubation for 1 hour and thereafter beads were washed and eluted. 410 µl IgG antibody were obtained. Purity was visualized with SDS-PAGE under reducing conditions. Harvest input (lane 1), harvest unbound (lane 2), and 3 µg purified IgG antibody (lane 3). The light and heavy chain of IgG migrates at 25 and 50 kDa, respectively

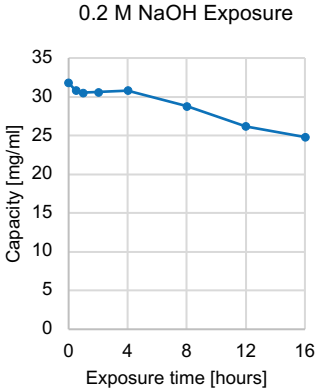


Fig 3 Exposure of MAGic™Beads mAb to 0.2 M NaOH. MAGic™Beads mAb was incubated with 0.2 M NaOH for various times. The beads were then regenerated with PBS and a standard capacity test was performed (incubating 50 µl MAGic™Beads mAb with rabbit IgG (~2 mg/ml in 1 ml PBS) for 60 minutes at room temperature). After 8 hours exposure, 90% of the total binding capacity remains.

12. Disclaimer

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

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13. Ordering information

Products	Quantity	Product No.
MAGic™Beads mAb	1 ml beads	1501
MAGic™Beads mAb	10 ml beads	1502
MAGic™Beads mAb	25 ml beads	1503
MAGic™Beads mAb	50 ml beads	1504
MAGic™Beads mAb	250 ml beads	1505
MAGic™Beads mAb	1 l beads	1506

Related products	Product No.
MAGic™Beads ACT	1300
MAGic™Accio LAB rack	2000
MAGic™Accio LAB cube	2100
MAGic™Accio PILOT 50	2200
MAGic™Accio PILOT 500	2300
MAGic™Accio PILOT 2000	2400

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