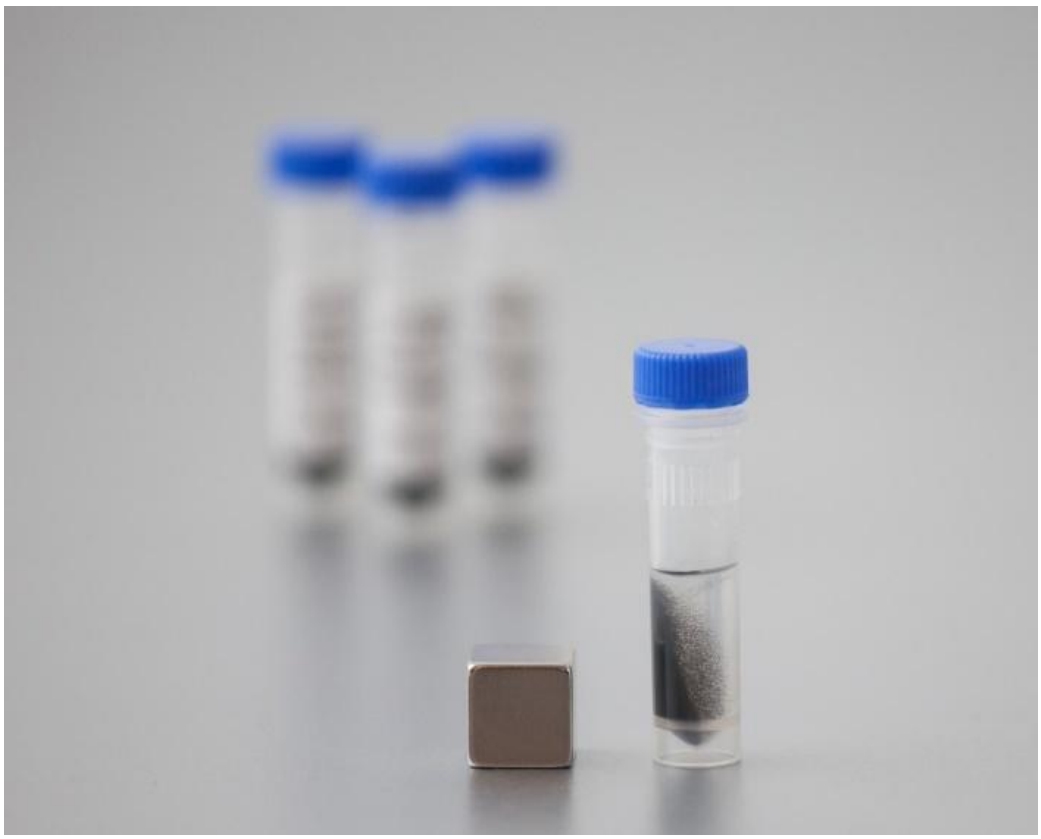
MAGic™ BioProcessing

DATA SHEET

MAGic™Beads Protein A



- Super-paramagnetic porous beads
- High binding capacity - 65 mg IgG/mL
- Alkali-stable up to 0.5 M NaOH
- Minimal non-specific interaction
- Negligible protein A leakage

MAGicBeads Protein A

MAGic™Beads Protein A provides a simple and effective system for small to medium scale 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 90 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. An alkali-stable recombinant protein A have sub-sequentially been coupled to this bead to produce MAGicBeads Protein A. The protein A have been expressed in *E. coli* using animal-free medium. The beads can be sanitized with up to 0.5 M NaOH. 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 A

Product	MAGicBeads Protein A, 10% bead suspension
Target molecule	Antibodies (IgG), bound via the F_c-region
Matrix	Super-paramagnetic porous agarose
Ligand	Alkali-resistant recombinant Protein A
Average particle size (DV50)[1]	90 µm
Particle size distribution	50–150 µm
Practical binding capacity[2]	45 mg IgG/mL settled beads
Maximum binding capacity[2]	65 mg IgG/mL settled beads
Chemical stability	Compatible with all standard aqueous buffers used for protein purification and with 0.5 M NaOH (pH 12), 0.1 M sodium citrate buffer (pH 3), 6 M guanidine-HCl, and 20% ethanol. Should not be stored at low pH for prolonged time
CIP stability	0.5 M NaOH
Storage	+2 to +8°C in 20% ethanol.
Protein A ligand leakage[5]	10–100 ng/mg IgG (10–100 ppm)

[1] The median particle size of the cumulative volume distribution
[2] Binding capacity at above 6 mg IgG/mL.
[3] Some antibodies may require different elution conditions.
[4] Data of product stability is continuously updated based on ongoing stability studies.
[5] 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.

Applications

Principle

MAGicBeads Protein 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 90 µm super-paramagnetic are beads based on crosslinked agarose.

High alkali stability

The alkali-stability of the beads have been tested by incubating the beads in 0.5 M sodium hydroxide (NaOH) in up to 25 hours at 22 °C, which equals 100 purification cycle with a contact time of NaOH of 15 minutes per cycle. The beads were tested for IgG binding capacity every 5 hours (see diagram below). After 25 hours of 0.5 M NaOH incubation, the beads had still 90% capacity left.

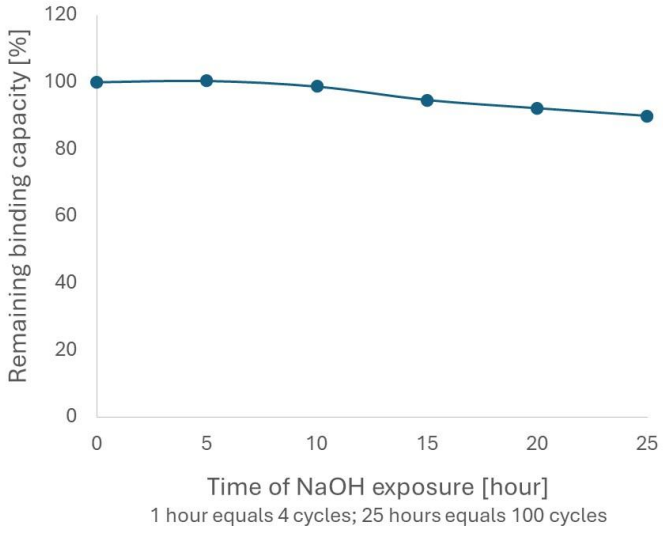


Figure 1. 90% of binding capacity remaining after 25 hours of 0.5 M NaOH exposure which equals 100 CIP cycles with a contact time of 15 minutes.

High binding capacity (BC)

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.

There is a positive correlation between binding capacity and antibody concentration.

For high IgG concentrations > 6 mg/mL:

- Maximum BC: 65 mg/mL (human IgG)
- Practical BC: 45 mg/mL (rabbit IgG)

Antibody binding concentration dependence - calculations

The capacity is as stated above 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).

Figure 2 and Table 2, demonstrates the practical binding capacity for Protein A beads 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 MAGicBeads Protein A, i.e. the optimal beads-target ratio to use. Insufficient Protein A beads will reduce binding capacity, and excess binding sites can amplify non-specific signals when sites are saturated.

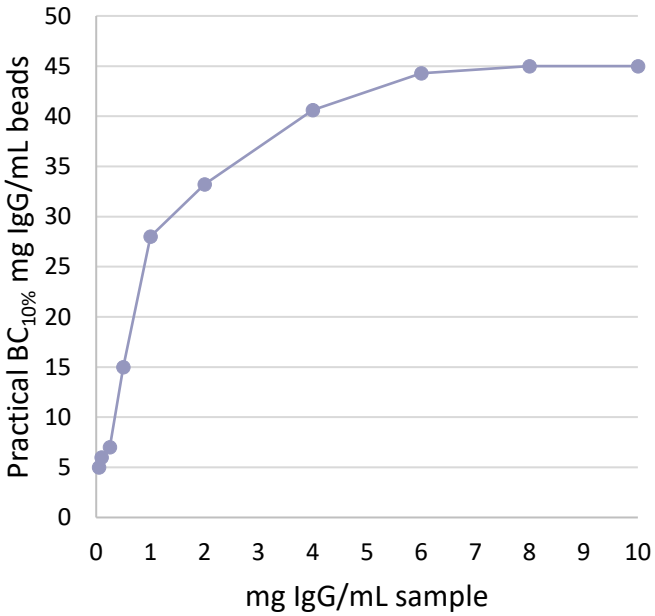


Figure 2. Practical binding capacity for IgG adsorption using magnetic Protein A beads, as exemplified with varies with different concentrations of antibody. Sample points show 90% adsorption at one hour. incubation per mL of beads.

Table 2. Practical binding capacity at different concentrations of rabbit IgG antibody per mL of Protein A magnetic beads 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

Super-paramagnetism

MAGicBeads are super-paramagnetic, where the 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.

High yields achieved in purifications from complex feeds

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

IgG1 expressed in Chinese Hamster Ovary (CHO) cells at a titer of 1.3 mg/mL were purified on MAGic beads Protein A.. Based on data in table 2, 464 µL 10% bead slurry was equilibrated in PBS and 1 mL of antibody feed was added. Suspension was incubated for an hour under stirring and after 3 wash steps, was eluted in 100 mM citrate buffer, pH 3. For yields from purifications on different MAGicBeads Protein A batches, see table 3 below.

Table 3. IgG1 elution yield from MAGicBeads Protein A after 1-hour adsorption. IgG1 was expressed in CHO cells at a titer of 1.3 mg/mL.

MAGicBeads Protein A batch	Eluted IgG1 yield (%)
Batch A	93
Batch B	94
Batch C	87
Batch D	91

The dynamic range for antibody titers was investigated by a purification setup with 3 different product concentrations ranging from 0.03 to 1.30 mg IgG per ml of cell culture, thus including conditions from low, medium high producer cell lines. Beads were added to 10 mL of POD filtered CHO cell culture expressing IgG in optimal ratios and purified in PBS buffer and eluted in 60 mM citrate in 2 steps, pH 3. The results for the purifications are shown in Table 4, and Figure 3 displays the yields as a function of IgG concentration in the CHO-cell cultures. High yields of 80% in the 1st elution fraction and nearly 100% in the pooled fractions were obtained for all the 3 investigated mAb concentrations spanning from low to high expression levels.

Table 4. Purification results from the 3 different IgG containing cell cultures obtained with a magnetic bead extraction system. First row in each category displays results in 1st elution fractions. Second row, 2nd elution fractions. Third row, all elution fractions pooled.

	0.027 mg IgG/ml	**0.40 mg IgG/ml**	**1.30 mg IgG/ml**
Recovered IgG	0.224 mg (1st elution)	3.14 mg	5.58 mg
	0.044 mg (2nd elution)	0.81 mg	1.60 mg
	0.267 mg (Total)	3.95 mg	7.18 mg
IgG concentration in eluate	0.447 mg/ml	3.14 mg/ml	5.58 mg/ml
	0.087 mg/ml	0.81 mg/ml	1.60 mg/ml
	0.267 mg/ml	1.97 mg/ml	3.59 mg/ml
Concentration factor	16.58	7.85	4.30
	3.23	2.02	1.23
	9.9	5.06	2.76

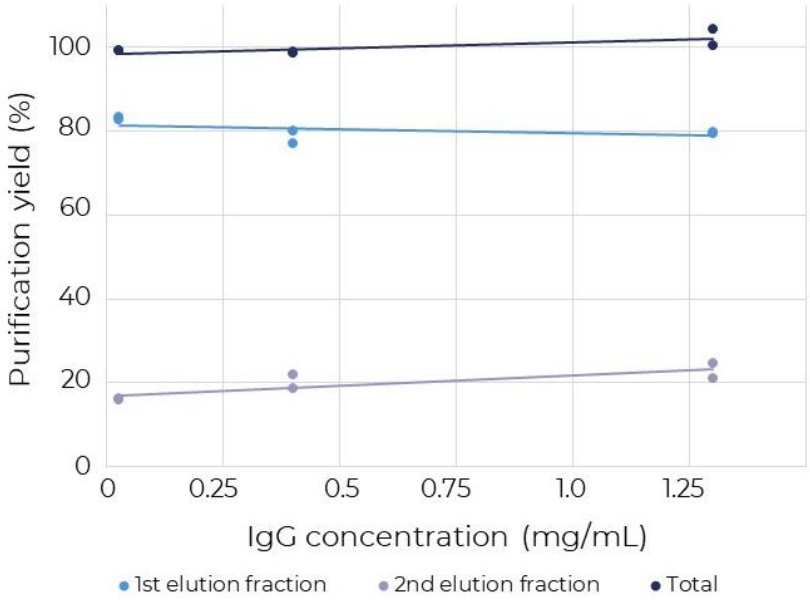


Figure 3. Purification yields from the 3 different CHO cell cultures expressing IgG at different expression levels at 0.027, 0.40 and 1.30 mg/mL.

See Figure 4 for purity profiles from a typical antibody purification using MAGicBeads Protein A as visualized with SDS-PAGE under reducing conditions.

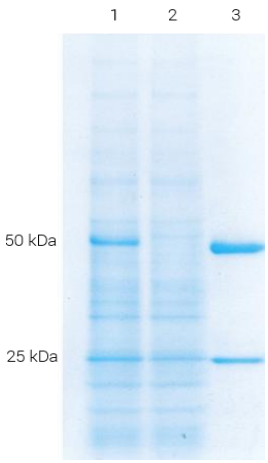


Figure 4. Feed input (lane 1), feed unbound (lane 2), and 3 µg purified IgG1 (lane 3). IgG1 light and heavy chain migrates at 25 and 50 kDa, respectively.

Scale-up

The beads are suitable for separations using appropriate magnetic separators, such as the MAGicAccio LAB (Product No. 2000, 2100), MAGicAccio PILOT 50 (Product No. 2200), MAGicAccio PILOT 500 (Product No. 2300), MAGicAccio PILOT 2000 (Product No. 2400) 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 A 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.

Ref: Nils A. Brechmann et al. 2024. Side-by-Side Economic Process Model for the Comparison and Evaluation of Magnetic Bead-Based Processes and Legacy Process for the Manufacturing of Monoclonal Antibodies. Processes.

Cleaning-in-place/ sanitation

During purification, impurities derived from the host cells such as proteins and nucleic acids may non-specifically bind to the beads. The impurities may reduce the performance and capacity of the beads. Regular cleaning (CIP) removes impurities and restores the capacity of the beads. Recommended CIP treatments are incubations with up to 0.5 M NaOH for a contact time of 15 minutes. A water cleaning step prior to and post the CIP step is always recommended.

Storage

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

Ordering information

Products	Quantity	Product No.
MAGicBeads Protein A	1 mL beads	1500
MAGicBeads Protein A	10 mL beads	1501
MAGicBeads Protein A	25 mL beads	1505
MAGicBeads Protein A	50 mL beads	1510
MAGicBeads Protein A	250 mL beads	1525
MAGicBeads Protein A	1 L beads	1550

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 Compact	3100
MAGicAccio PROCESS Maxi	3400

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