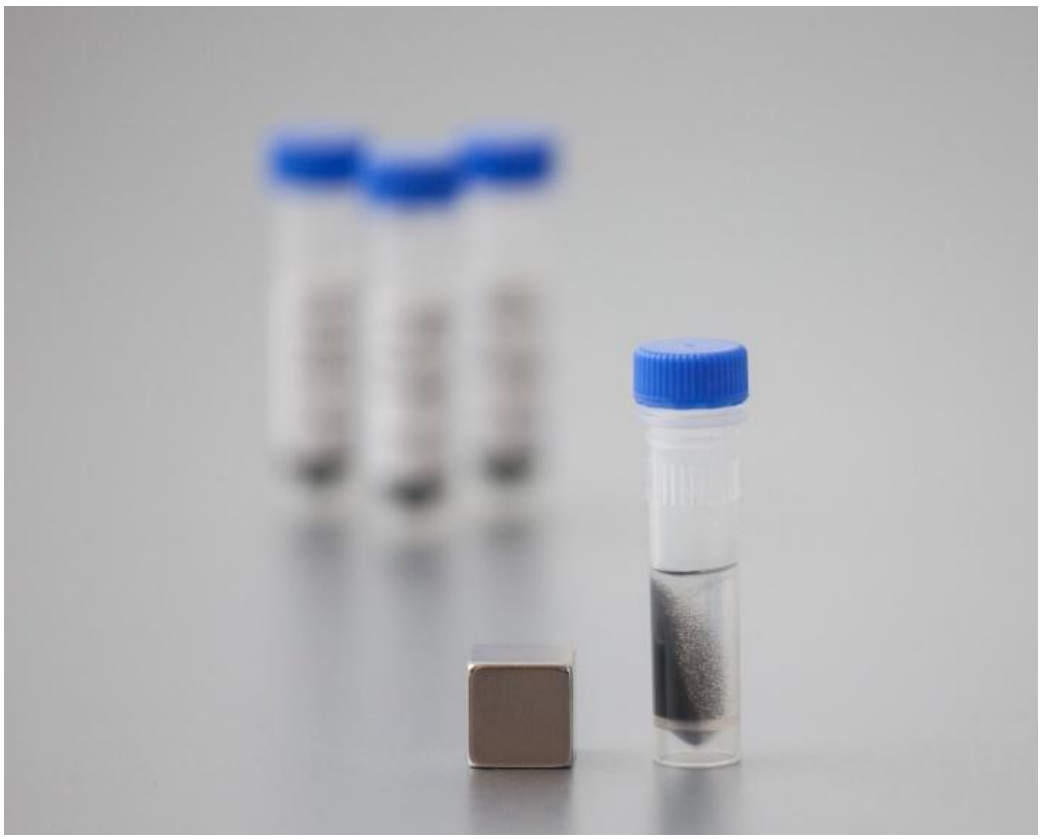
mAGic™
BioProcessing

DATA SHEET

MAGicBeads Protein A+



- Super-paramagnetic porous beads
- High binding capacity – 55 mg/mL
- Alkali-stable up to 0.5 M NaOH
- Minimal non-specific interaction

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 55 µm) with an optimal pore size for antibodies, and 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 has subsequently been coupled to this bead to produce MAGicBeads Protein A+. 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-stable recombinant Protein A
Average particle size (DV50)[1]	55 µm
Maximum binding capacity[2, 3]	~55 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 with 0.5 M NaOH, and 20% ethanol. Not compatible with citrate buffer. Should not be stored at low pH for a prolonged time
CIP stability	0.5 M NaOH
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 measured 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 A+ is a magnetic affinity separation medium 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 beads are based on crosslinked agarose.

Super-paramagnetism

MAGicBeads are super-paramagnetic beads, 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 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 Figure 1). After 25 hours of 0.5 M NaOH incubation, the beads had still 87% capacity left. One hour equals 4 CIP cycles with a contact time of 15 minutes, 25 hours equals 100 CIP cycles.

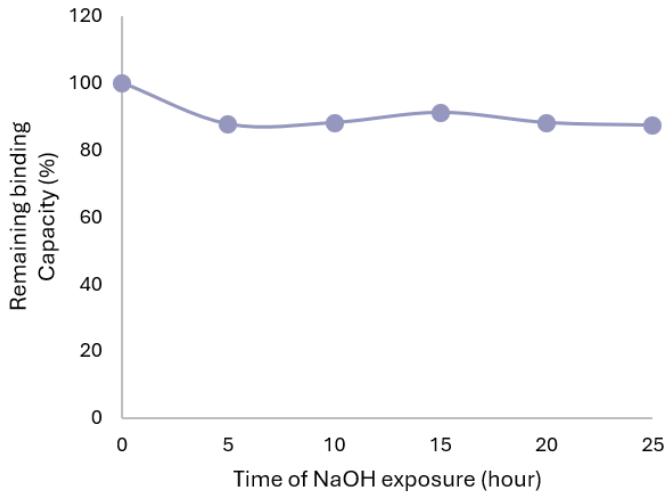


Figure 1. 87% of binding capacity remaining after 25 hours of 0.5 M NaOH exposure which equals 100 CIP cycles with a contact time of 15 minutes.

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 ≥ 5 mg/mL:

- Maximum BC: ~55 mg/mL (human IgG)
- Practical BC: ~45 mg/mL (human IgG)

Practical binding capacity - calculations

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 target-to-bead ratio can be calculated. See the instructions for these calculations.

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

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 2. Maximum binding capacities were determined as a function of IgG concentration, and the results are presented in Figure 2.

Table 2. Experimental condition for binding studies to investigate the correlation between IgG titer and binding capacities. Last column displays the maximum binding capacities measured under the specified conditions.

#	IgG concentration (mg/mL)	Reaction volume (mL)	Bead volume (µL)	Maximum binding capacities (mg IgG/mL)
1	5	1	50	54
2	2	1	50	35
3	0.1	2	10	16

The data demonstrates a positive correlation between antibody titer and binding capacity. At a high titer of 5 mg/mL, a binding capacity of 54 mg IgG/mL beads wass observed. In contrast, at a lower antibody titer at 0.1 mg /mL the binding capacity decreased to 16 mg IgG/mL beads.

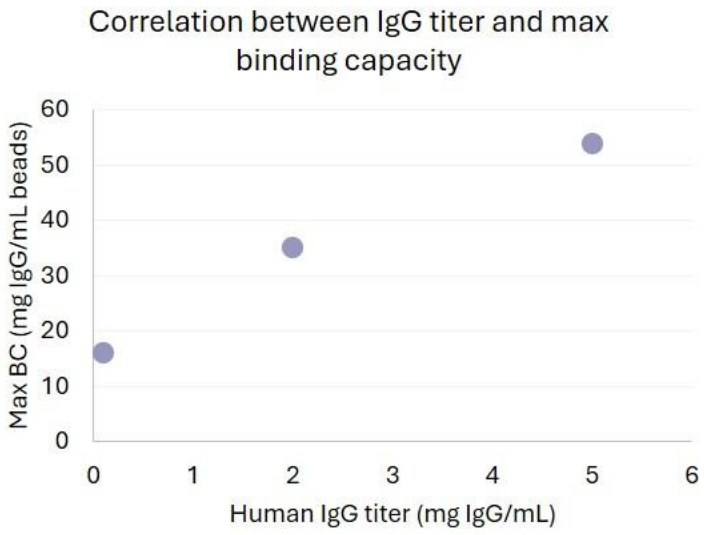


Figure 2. Maximum binding capacities of human IgG (pure) to MAGicBeads Protein A+ at increasing titers ranging from 0.1 mg/mL up to 5 mg/mL.

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 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 was purified on MAGicBeads Protein A+. 464 µL of 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 three wash steps, the antibody was eluted in 100 mM glycine buffer, pH 2.7 in two consecutive elution fractions. For recoveries from purifications on different MAGicBeads Protein A+ batches, see Table 3 below.

Table 3. IgG1 elution recoveries 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 recovery (%)
Batch A	87
Batch B	91
Batch C	88

To achieve high IgG recoveries exceeding 80%, two consecutive elution steps should be performed. Table 4 and Figure 3 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 B), approximately 80% of the total IgG was recovered in the first elution fraction, while pooling the two elution fractions increased the overall yield to more than 90%.

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 260/280 ratio for the purifications were <0.65, indicating a high degree of purity.

Table 4. Purification results from an IgG-containing cell culture (Batch B) 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 fraction pooled.

	Feed: 1 mL of 1.30 mg IgG/mL
Recovered IgG (mg)	1.02 (1st elution) 0.16 (2nd elution) 1.18 (Total)
Yield (%)	78.5 (1st elution) 12.5 (2nd elution) 91 (Total)

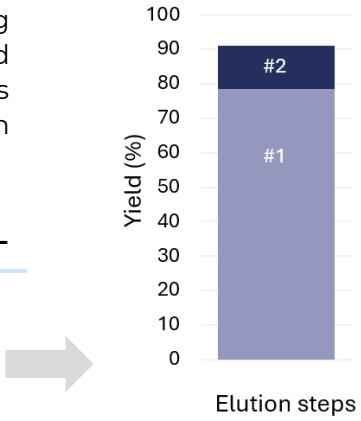


Figure 3. IgG recovery ratios in the two elution fractions.

Yields dependent on pH

The interaction between antibodies and Protein A 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, low-titer human IgG (0.1 mg IgG/mL) bound to MAGicBeads Protein A+ was eluted using 100 mM glycine buffers with pH values ranging from 2.5 to 3.0. As expected, the elution efficiency was highest at pH 2.5, resulting in an approximately 15% 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.

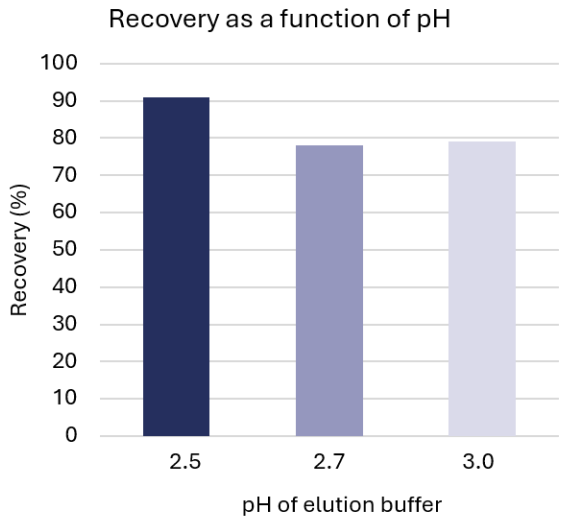


Figure 4. Recovery as a function of elution pH. Recoveries for human IgG bound to MAGicBeads Protein A+ are shown following elution with 100 mM glycine buffer at pH 2.5, 2.7, and 3.0.

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 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	1900
MAGicBeads Protein A+	10 mL	1901
MAGicBeads Protein A+	50 mL	1905
MAGicBeads Protein A+	100 mL	1910
MAGicBeads Protein A+	250 mL	1925
MAGicBeads Protein A+	500 mL	1950

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

MAGIC BIOPROCESSING
Virdings Allé 28
SE-754 50 Uppsala, Sweden

Email: info@magicbioprocessing.com
Web: https://magicbioprocessing.com