



Product no WHM-L086
Absolute Mag™ Protein A
Product Manual

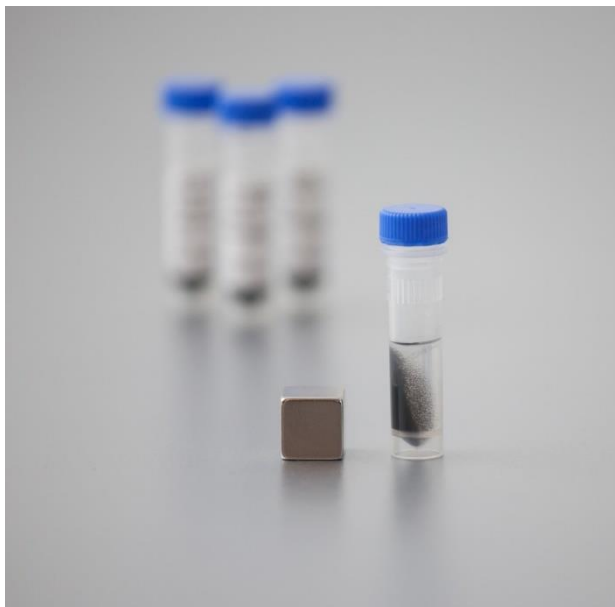


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1. General information

Absolute Mag™ Protein A consists of super-paramagnetic agarose beads covalently coupled with recombinant protein A. This product is an excellent tool in applications involving capture of antibody from different species and subclasses. Suitable applications include purification of IgG antibodies from serum and ascites, depletion of IgG from serum, and various immunosorbent techniques, such as immunoprecipitation.

The Absolute Mag™ Protein A magnetic agarose beads show outstanding magnetic behavior and are easily attracted to external magnets, allowing separation within seconds. The agarose matrix minimizes nonspecific binding of proteins due to its hydrophilic nature. The black beads are easily observed by the naked eye, making them easy to collect. The beads do not aggregate.

The maximum binding capacity is 6 mg of human or rabbit IgG per ml bead suspension (60 mg/ml settled beads). In 1 hour, efficient capture of antibodies with high yield and purity is obtained. The beads can be reused multiple times.

The quantity of beads can easily be scaled up or down to match antibody concentration and sample volumes. The beads are suitable for separations from µl to ml scale using appropriate magnetic separators, such as our Absolute Mag™ MagSep series.

2. Antibody affinity to protein A

Table 1. Binding strengths for protein A

Species	Subclass	Affinity	Species	Subclass	Affinity
Human	IgG ₁	++	Rabbit		++
	IgG ₂	++	Hamster		+
	IgG ₃	-	Guinea Pig		++
	IgG ₄	++	Bovine		+
	IgA	+	Horse		+
	IgD	+	Sheep		+/-
	IgE	+	Goat		+/-
	IgM	+	Pig		++
Mouse	IgG ₁	+	Chicken	IgY	-
	IgG _{2a}	++	Antibody fragments		Affinity
	IgG _{2b}	++	Human	Fab	+
	IgG ₃	+		F(ab') ₂	+
	IgM	+/-		scFv	+
Rat	IgG	++		Fc	+
	IgG ₁	+/-		κ	-
	IgG _{2a}	+/-		λ	-
	IgG _{2b}	+/-			
	IgG _{2c}	+/-			
	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 Absolute Mag™ Protein A

Matrix	Super-paramagnetic agarose
Product	Absolute Mag™ Protein A, 10% bead suspension
Ligand	Recombinant protein A
Particle size	45–165 µm
Binding capacity ¹	4 mg rabbit IgG/ml bead suspension (40 mg IgG/ml settled beads)
Max. binding capacity ²	6 mg human IgG/ml bead suspension
Binding conditions	PBS (137 mM NaCl, 2.7 mM KCl, 10 mM phosphate, pH 7.4)
Elution conditions	60 mM citrate pH 3.0 (Note: low pH glycine not suitable)
Storage conditions	+2 to +8 °C in PBS with 20% ethanol
Stability information ³	Stable for at least 24 months
Protein A ligand leakage ⁴	6 ng/mg of eluted IgG (6 ppm)
Reusability	Can be reused multiple times ⁵

¹ 90% binding of rabbit IgG (2 mg/ml) after 60 min reaction.

² Determined in an overloading test at 5 mg IgG/ml after 60 min reaction.

³ Data of product stability is continuously updated based on ongoing stability studies.

⁴ Protein A ligand leakage in the acidic elution fraction after 15 min contact time at room temperature. The leakage was determined by a Protein A ELISA kit (#03-96) from Immun System I.M.S AB, Sweden.

⁵ Not reusable when performing immunoprecipitations for, e.g., SDS-PAGE.

4. Material supplied

- Absolute Mag™ Protein A supplied as a 10% bead suspension in PBS with 20% ethanol. 1 ml 10% bead suspension contains 100 µl beads.
- Neodymium cube magnet (12 mm) suitable for separations in 0.5–5 ml vials.

5. Additional materials needed

- **Binding/Washing buffer** – For coupling of antibodies to beads and for washing, use PBS (137 mM NaCl, 2.7 mM KCl, 10 mM phosphate, pH 7.4 or similar recipe, e.g., 150 mM NaCl, 15 mM phosphate, pH 7.4).
- **Elution buffer** – For release of antibodies from beads, use 60 mM citrate pH 3.0 (low pH glycine not suitable).
- **Neutralization buffer** – To neutralize eluted antibodies use, 2 M Tris-HCl, pH 9.0
- **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 vial can also be used.
- **Magnetic separator** – For separations in volumes larger than 5 ml use the Absolute Mag™ MagSep 15/50 separator (Product No. 3001) or the Absolute Mag™ MagSep 500 separator (Product No. 4001).
- Additional vials/tubes, pipettes, and pipette tips

6. Handling instructions

Use approximately 500 µl 10% bead suspension (50 µl settled beads) for each 1–2 mg antibody to isolate.

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.

Separation of magnetic beads

- The provided neodymium cube magnet can be used to collect the beads from liquid volumes up to 5 ml. For volumes from 5 ml up to 50 ml use the Absolute Mag™ MagSep 15/50 separator (Product No. 3001) or the Absolute Mag™ MagSep 500 separator for volumes up to 500 ml (Product No. 4001; Section 12).
- 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 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.

7. Product operation

Intended use

- This product is intended for purification of antibodies from serum and ascites fluid samples, IgG serum depletion, and immunoprecipitation.
- The product is not intended for applications in cell culture media containing high levels of free thiols.

Binding

- Absolute Mag™ Protein A bind immunoglobulins with a various affinity (Table 1), in the range of pH 6 to 8. It is recommended to use PBS (137 mM NaCl, 2.7 mM KCl, 10 mM phosphate, pH 7.4) as the binding and washing buffer.
- The high binding capacity of beads is reached within 1 hour of incubation in serum and ascites fluid samples (Section 10). Uptake rate is dependent on concentration of IgG. Therefore, incubation time and/or bead volume can be optimized.

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 stronger 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 60 mM citrate, pH 3.0, for most antibodies.
- The elution buffer may need optimization, as different immuno-globulins eluate at different pH values depending on species and subclass. Some immunoglobulins are also more sensitive towards low pH (acid-labile). Optimized elution buffers include, e.g., 60–200 mM citrate with pH 2.6–3.4. For high pH options, consult current literature and/or ready-made elution buffers from other commercial sources.
- For monoclonal antibodies, always analyze the elution efficiency from the beads and perform a functional characterization of the eluted and desalted antibody.
- 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

- If a purified monoclonal antibody has a tendency to precipitate, the pH of the solution might be close to the pI of the antibody; a common cause of precipitation for many proteins. In such cases, optimize by trying different buffer pH options.

- The adsorbed antibodies are generally eluted within 1 min of mixing with elution buffer.
- Normally 88% of bound material is found in the first elution fraction, 10% in the second, and 2% in the third (in-house data for 10 bead volume elution fractions).

Note: Bead volume is the volume of settled beads, i.e., 10% of the delivered bead suspension volume. 500 µl bead suspension corresponds to a 50 µl bead volume.

Regeneration

- The beads can normally be reused multiple times without loss in binding capacity or selectivity.
- To regenerate the beads, wash a minimum of three times with 10 bead volumes elution buffer and twice with 10 bead volumes binding buffer.
- When reusing beads, it is recommended to use the particles for purification of the same antibody in order 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.
- Use only cleaning agents with a pH of 2–11.

Storage

- The Absolute Mag™ Protein A beads should be stored as a 10% bead suspension at +2 to +8 °C in PBS containing 20% ethanol.

Optimization

The general recommendations in this instruction 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
- Number of washes
- Elution time

8. 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 with a quick spin in a micro-centrifuge.
- 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 incubation time of antibodies to beads depending on sample source and antibody concentration, especially during IgG depletion application.
- If the antibody is sensitive to the low pH during elution, optimize elution conditions to identify the highest pH usable for efficient elution. Also, always neutralize and/or desalt the eluted fraction. Minimize the antibody's exposure to extreme pH as much as possible.
- When reusing beads, it is recommended to use the beads for purification of the same antibody in order to avoid any cross-contamination between purification runs.

9. General protocols

The product is intended for applications involving isolation of antibodies from serum and ascites fluid samples.

When designing an experiment, consider the affinity of the antibody to protein A (Table 1).

Purification of 1–2 mg IgG from serum or ascites

Bead preparation

1. Mix bead suspension thoroughly by manual inversion of the bead suspension vial.
2. Dispense 500 µl of bead suspension in a test tube.
3. Remove liquid by magnetic separation.
4. Resuspend beads in 500 µl PBS.
5. Remove the liquid.
6. Resuspend beads in 500 µl PBS.

Sample application

7. Add 250–500 µl serum or ascites to the beads.
8. Incubate with continuous mixing using an end-over-end mixer for 30–60 min.
9. Remove the liquid.

Washing

10. Add 500 µl binding buffer, resuspend the beads, and mix for 30 sec by manual inversion.
11. Remove the liquid.
12. Perform steps 10 and 11 a total of three times.

Elution

13. Add 300–500 µl of elution buffer (60 mM citrate, pH 3.0).
14. Resuspend the beads and mix for 1 min by manual inversion.
15. Remove and collect the elution fraction. Generally, 85–90% of bound antibody is found in the first elution fraction.
16. Repeat elution step if necessary.
17. Regenerate beads and resuspend in storage solution (Section 7).

Depletion of IgG from 50 µl serum

Bead preparation

1. Mix bead suspension thoroughly by manual inversion of the bead suspension vial.

2. Dispense 200 µl of bead suspension in a test tube.
3. Remove liquid by magnetic separation.
4. Resuspend beads in 500 µl PBS.
5. Remove the liquid.
6. Resuspend beads in 700 µl PBS.

Sample application

7. Add 50 µl serum to the beads.
8. Incubate with continuous mixing with an end-over-end mixer for 60 min.
9. Separate beads from the serum sample by magnetic separation. Collect the supernatant, which represents the depleted serum sample.

10. Performance

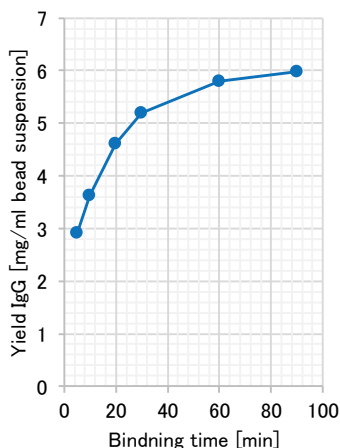


Fig 1. Dependency of antibody binding capacity on binding time for Absolute Mag™ Protein A. 500 μ l pre-washed bead suspension (50 μ l beads) was mixed with 500 μ l human serum. The beads were allowed to bind IgG for 5, 10, 20, 30, 60, and 90 min, before washing and elution. The graph shows the amount of purified IgG obtained per ml of bead solution at the different binding times. Most of the binding capacity is reached within one hour, corresponding to 6 mg IgG/ml of bead suspension.

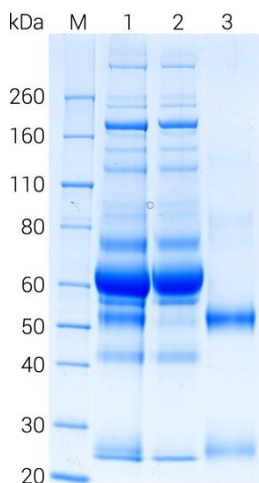


Fig 2. Depletion and SDS-PAGE analysis of human IgG from serum using Absolute Mag™ Protein A. The content of 200 μ l 10% bead suspension (20 μ l beads) was mixed with 50 μ l human serum and 700 μ l PBS for 60 min. Beads were collected, washed, and eluted using a handheld neodymium cube magnet as separator. Two elution fractions of 200 μ l each, gave a total amount of 352 μ g eluted material. Samples were separated on SDS-PAGE under reducing conditions and stained using colloidal Coomassie. Lane M: size marker, lane 1: input serum, lane 2: depleted serum, lane 3: eluted IgG antibody (2 μ g).

11. Disclaimer

The product is not fully tested and is not intended for human use. For *in vitro* and research use only