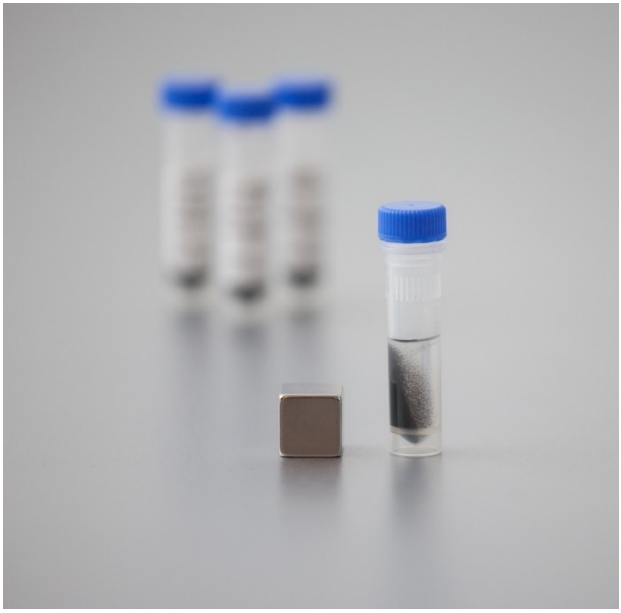
LOABeads™ MabBind A

Product Manual



Lab on a Bead AB

Revision date 2016-09-05

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Please read through this manual carefully before using LOABeads MabBind A.

Intended use

This product is intended for purification of monoclonal antibodies from cell culture samples.
For research use only.

1. General information

LOABeads MabBind A consists of super-paramagnetic agarose beads covalently coupled with a standard recombinant protein A ligand. 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, or for use in immunoprecipitation experiments, please choose LOABeads Protein A (Product No. 1001).

The LOABeads MabBind A particles show outstanding magnetic behavior and 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 binding capacity is 3 mg of human or rabbit IgG per ml 10% bead suspension (30 mg/ml settled beads), but the capacity can vary among IgG isoforms from different species. The beads can be used multiple times without noticeable loss of binding selectivity and capacity.

LOABeads MabBind A provides a simple and effective system for small to medium scale isolation of antibodies using magnetic separation techniques. The quantity of beads needed can easily be scaled up or down to match the antibody concentration and sample volume. The beads are suitable for separations from µl to high ml scale using appropriate magnetic separators, such as the LOABeads MagSep 15/50 (Product No. 3001) and LOABeads MagSep 500 (Product No. 4001).

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}	++			
	IgG_{2b}	++	Antibody fragments		Affinity
	IgG_3	+	Human	Fab	+
	IgM	+/-		F(ab')2	+
Rat	IgG	++		scFv	+
	IgG_1	+/-		Fc	+
	IgG_{2a}	+/-		κ	-
	IgG_{2b}	+/-		λ	-
	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 LOABeads MabBind A

Matrix	Super-paramagnetic agarose
Product	LOABeads MabBind A, 10% bead suspension
Ligand	Recombinant protein A
Particle size	45–165 µm
Binding capacity[1]	3 mg rabbit IgG/ml 10% bead suspension (30 mg IgG/ml settled beads)
Binding conditions	Directly in cell culture media, pH 6–8
Elution conditions[2]	60 mM citrate pH 3.0 Note, low pH glycine not suitable
Storage	+2 to +8°C in PBS with 20% ethanol.
Stability[3]	12 months
Protein A ligand leakage[4]	10–100 ng/mg IgG (10–100 ppm)
Reusability	Can be reused multiple times

1 Binding capacity was determined by incubating 500 µl 10% LOABeads MabBind A (50 µl beads) with rabbit IgG (~2 mg/ml in 1 ml PBS) for 60 minutes at room temperature. Binding capacity is obtained with a yield of 90% or more under the conditions specified above.

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

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

5. Additional materials needed

- Washing buffer – For washing of beads, use PBS (137 mM NaCl, 2.7 mM KCl, 10 mM phospate, 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 60 mM citrate, pH 3.0.
- 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 the vial can also be applied.
- Magnetic separator – For separation of volumes larger than 5 ml, use LOABeads MagSep 15/50 (Product No. 3001) for volumes up to 50 ml, or LOABeads MagSep 500 (Product No. 4001) for volumes up to 500 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

- 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 it is recommended to use the LOABeads MagSep 15/50 separator. Use the LOABeads MagSep 500 separator for volumes up to 500 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, the time for adsorption is increased when purifying antibodies of low concentrations. As an alternative, the amount of beads can be increased to accelerate the binding (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 starting material. See Section 8 for advice.

Binding

- The LOABeads MabBind 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 phospate, 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 60 mM citrate, pH 3.0, 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. 500 µl bead suspension corresponds to 50 µl 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. Low pH glycine is not suitable as elution buffer. 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 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 LOABeads MabBind A 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. Bead input and binding time

- The binding kinetics between beads and antibody depends on the concentration of antibodies in the sample. Variation between different monoclonal antibodies also affect kinetics. Optimization of binding conditions for a specific antibody can be performed on small sample volumes, in the range of 1–10 ml cell culture media, depending on the antibody concentration. Scaling up to larger sample volumes, usually do not affect purity and yield negatively.

- In practice, to achieve an acceptable yield in a limited time, the Load (L) needs to be adjusted accordingly (Table 3).
- The Load determines the amount of beads used, Bead Input (BI), for a specific purification.

$$BI = \frac{m}{BC * L}$$

BI = Bead Input (amount of 10% bead suspension to add in ml)
m = total amount of antibody in sample in mg
BC = Binding Capacity (3 mg/ml bead suspension)
L = Load (ratio of actual binding capacity to theoretical binding capacity; Table 3)

- Example: A 250 ml sample contains IgG at 0.1 mg/ml and a total of 25 mg IgG. Use Table 3 to determine load and binding time.

  A Load of 0.33 gives a Bead Input of 26 ml 10% bead suspension with 3 hours binding.

$$BI = \frac{25mg}{3mg/ml * 0.33} = 25.7ml$$

  A Load of 0.5 gives 17 ml 10% bead suspension with 6 hours binding.

$$BI = \frac{25mg}{3mg/ml * 0.5} = 16.7ml$$

Table 4. Load and binding times relative IgG concentration to reach ~90% yield.

IgG, mg/ml	Load	Binding time, hour
<0.25[1]	0.1	6
0.25	0.17	3
	0.08	2
0.05	0.17	2
	0.08	1
0.10	0.5	6
	0.33	3
0.50	0.5	3
	0.42	2
1.0	1.0	3
	0.5	1
>1.0	1.0	1–3

[1] Requires optimization of Bead Input (BI) and binding time.

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 LOABeads MagSep 15/50 and/or LOABeads MagSep 500 separators, please read the product manuals for those for further instructions.

Bead pre-treatment

1. Calculate the amount of beads to use, Bead Input (BI), for your specific purification (Section 8).
2. Mix bead suspension thoroughly.
3. Dispense beads 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 in 1–3 bead volumes of sample media and transfers to the main sample container.
9. Incubate with continuous mixing during the adsorption step.
10. Remove the liquid after the set adsorption time. Save sample 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 12 and 13 a total of three times.

Elution

14. Add 1–10 bead volumes of elution buffer.
15. Resuspend the beads and mix for 1 min.
16. Remove and collect the elution fraction. Generally, 50–90% 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: 500 µl bead suspension corresponds to 50 µl bead volume. 50 µl bead volume is the same as 50 µl 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 in order 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

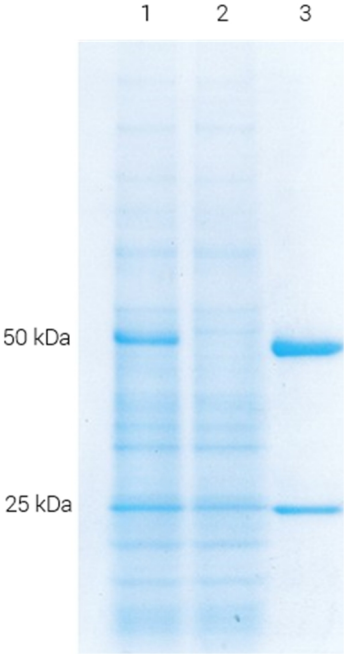

Fig 2. Example of Load usage. A 20 ml CHO cell culture harvest with a monoclonal IgG antibody titer of 0.02–0.025 mg/ml, a total amount of 0.5 mg IgG was used, together with a Load of 0.17 (obtained from Table 4), for calculations with the *BI*-formula. The calculated amount of 10% bead suspension to use, was 0.98 ml. For the purification, 1.0 ml bead suspension were used at an adsorption step for 2 hours, whereafter beads were washed and eluted. 410 µg purified 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

12. Disclaimer

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

13. Ordering information

Products	Quantity	Product No.
LOABeads MabBind A	1 ml beads	1004-1ml
LOABeads MabBind A	5 ml beads	1004-5ml

Related products	Quantity	Product No.
LOABeads Protein A	2x1 ml 10% beads	1001-2ml
LOABeads Protein A	5x1 ml 10% beads	1001-5ml
LOABeads Protein A	10 ml 10% beads	1001-10ml
LOABeads AffiAmino	2x1 ml 10% beads	1003-2ml
LOABeads AffiAmino	5x1 ml 10% beads	1003-5ml
LOABeads AffiAmino	10 ml 10% beads	1003-10ml
NdFeB cube magnet	1	2001
LOABeads MagSep 15/50	1	3001
LOABeads MagSep 500	1	4001

LAB ON A BEAD AB

Postal address:
Toftebergsvagen 7
SE-442 75 Lycke, Sweden

Visiting address:
Virdings Allé 28
SE-754 50 Uppsala, Sweden

Email: info@labonabead.se
Web: www.labonabead.se