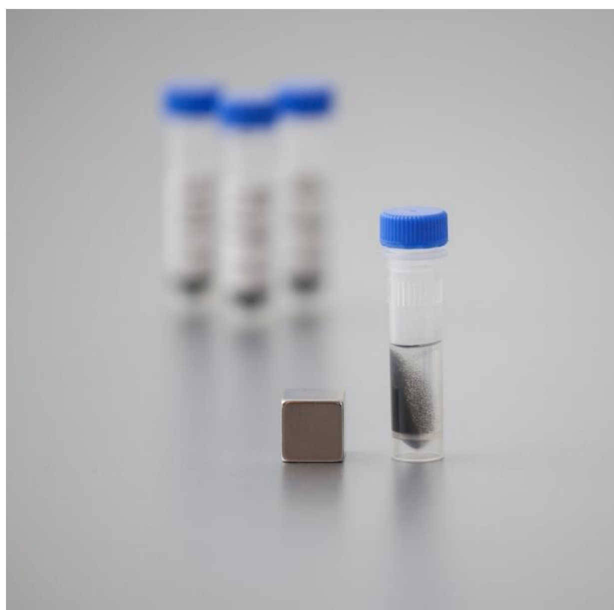
lab on a bead®
Beadsurface Biotechnology

LOABeads™ SerAscA

Magnetic bead purification of antibodies
Capacity 60 mg IgG/ml

Product Manual



Lab on a Bead AB

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Please read through this manual carefully before using LOABeads SerAscA.

Intended use

LOABeads SerAscA are intended for purification of antibodies from serum and ascites fluid samples and for IgG serum depletion.

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

1. General information

LOABeads SerAscA possess an extra-high capacity and is used for purification of antibodies, with affinity to protein A, from serum and ascites fluid, as well as depletion of IgG from serum, and various immunosorbent techniques. For purification of antibodies from cell culture harvests, we recommend LOABeads PrtA, AlkaliA, or PrtG, since LOABeads SerAscA is sensitive to additives in some cell culture broths. For IgG antibodies that binds poorly to protein A, please consider using a protein G-containing bead (see Table 1 for affinities).

The LOABeads SerAscA particles are produced using a proprietary method, which provides superior binding capacity. The super-paramagnetic agarose beads 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 60 mg of human or rabbit IgG per ml settled beads. In one-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 LOABeads MagSep series.

2. Antibody affinity to protein A and protein G

Table 1. Binding strengths for protein A

		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 LOABeads SerAscA

Matrix	Super-paramagnetic agarose
Product	LOABeads SerAscA, 10% bead suspension
Ligand	Recombinant protein A
Particle size	45–165 µm
Binding capacity[1]	60 mg human IgG/ml settled beads
Static binding capacity	67 mg IgG/ml
Binding conditions	PBS (137 mM NaCl, 2.7 mM KCl, 10 mM phosphate, pH 7.4)
Elution conditions	100 mM citrate pH 2.8 (Note: low pH glycine not suitable)
Storage conditions	+2 to +8°C in PBS with 20% ethanol
Stability information[2]	Stable for at least 24 months
Protein A ligand leakage[3]	6 ng/mg of eluted IgG (6 ppm)
Reusability	Can be reused multiple times[5]

1 Binding capacity at 5 mg IgG/ml sample.
2 Data of product stability is continuously updated based on ongoing stability studies.
3 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.

4. Material supplied

- LOABeads SerAscA supplied as a 10% bead suspension in PBS with 20% ethanol. 10 ml 10% bead suspension contains 1 ml beads.

5. Additional materials needed

- Binding/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., 150 mM NaCl, 15 mM phosphate, pH 7.4).
- Elution buffer – For release of antibodies from beads, use 100 mM citrate pH 2.8 (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 – LOABeads MagSep5 (Product No. 2100) is suitable for separations in 0.5–5 ml volumes. For separations in volumes larger than 5 ml use the LOABeads MagSep50 separator (Product No. 2200) or the LOABeads MagSep500 separator (Product No. 2300).
- Additional vials/tubes, pipettes, and pipette tips

6. Handling instructions

Use approximately 50 µl settled beads (500 µl 10% bead suspension) 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

- LOABeads MagSep5 (Product No. 2100) 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 LOABeads MagSep50 separator (Product No. 2200) or the LOABeads MagSep500 separator for volumes up to 500 ml (Product No. 2300; Section 11).
- 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 and IgG serum depletion.
- The product is not suitable for applications in cell culture media containing high levels of free thiols.
- The product is not suitable for immunoprecipitations.

Binding

- LOABeads SerAscA bind immunoglobulins with a various affinity (Table 1), in the range pH 6–8. 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 the 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 100 mM citrate, pH 2.8, for most antibodies. Low pH glycine buffers are not recommended.
- The elution buffer may need optimization, as different immunoglobulins 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 from ascites, 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 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. 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 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 SerAscA 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 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 50 mg IgG from serum or ascites

The amount of sample to be used depends on the species it originates from, due to varying IgG concentrations.

Bead preparation

1. Mix bead suspension thoroughly by manual inversion of the bead suspension vial.
2. Dispense 10 ml of 10% bead suspension (1 ml beads) in a test tube.
3. Remove liquid by magnetic separation.
4. Resuspend beads in 10 ml PBS.
5. Remove the liquid.
6. Resuspend beads in 10 ml PBS.

Sample application

7. Add 3–5 ml serum or 5–10 ml 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 10 ml PBS, resuspend the beads, and mix for 1 min by manual inversion.
11. Remove the liquid.
12. Perform steps 10 and 11 at least three times.

Elution

13. Add 10 ml of elution buffer (100 mM citrate, pH 2.8).
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. Neutralize the eluted antibody with, e.g., 1 ml 2 M Tris-HCl, pH 9.0.
18. Concentrate the product and/or change buffer, e.g., using a spin filter device (cutoff 30 kDa), gel filtration, or by dialysis.
19. Regenerate beads and resuspend in storage solution (Section 7).

Depletion of IgG from 1 ml serum

Bead preparation

1. Mix bead suspension thoroughly by manual inversion of the bead suspension vial.
2. Dispense 5 ml of 10% bead suspension in a test tube.
3. Remove liquid by magnetic separation.
4. Resuspend beads in 5 ml PBS.
5. Remove the liquid.
6. Resuspend beads in 5 ml PBS.

Sample application

7. Add 1 ml 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. Wash and regenerate beads. Collect eluted IgG if desired.

10. Performance

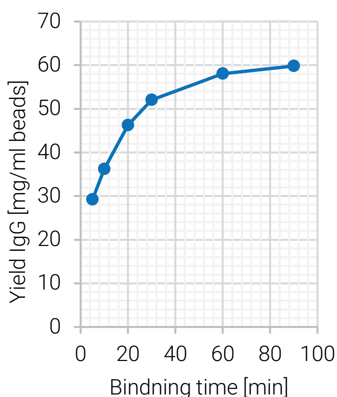

Fig 1. Dependency of antibody binding capacity on binding time for LOABeads SerAscA. 50 µl pre-washed beads was mixed with 500 µl human serum. Beads were allowed to bind IgG for 5, 10, 20, 30, 60, and 90 min, before washing and elution. The graph shows the amount of purified IgG obtained per ml of beads at the different binding times. Most of the binding capacity is reached within one hour, corresponding to 60 mg IgG/ml sample.

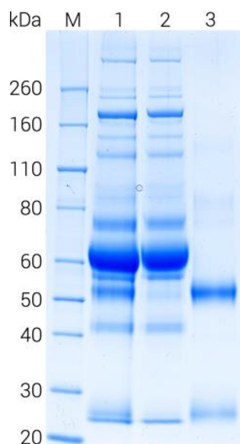

Fig 2. Depletion and SDS-PAGE analysis of human IgG from serum using LOABeads SerAscA. 20 µl beads was mixed with 50 µl human serum and 700 µl PBS for 60 min. Beads were collected using LOABeads MagSep5, washed, and eluted. Two elution fractions of 200 µl each, gave a total amount of 352 µg eluted material. 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 µg).

11. Contact and ordering information

Products	Quantity	Product No.
LOABeads SerAscA	1 ml beads	1201
LOABeads SerAscA	5 ml beads	1202
LOABeads SerAscA	25 ml beads	1203

Related products	Product No.
LOABeads PrtA	1100
LOABeads AffiActive	1300
LOABeads IP-A	1400
LOABeads AlkaliA	1500
LOABeads PrtG	1600
LOABeads IP-G	1700
LOABeads MagSep5	2100
LOABeads MagSep50	2200
LOABeads MagSep500	2300

LAB ON A BEAD AB

Postal address:
Medicinaregatan 8A
SE-413 90 Göteborg, Sweden

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

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

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