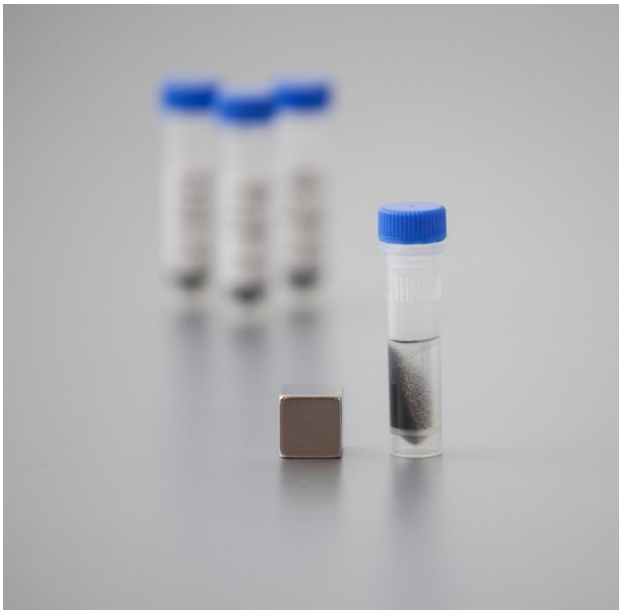
LOABeads™ IP30

Product Manual



Lab on a Bead AB

Revision date 2017-11-28

Table of Contents

1. General information

LOABeads IP30 consists of super-paramagnetic agarose beads covalently coupled with a standard recombinant protein A ligand. This product is intended for immunoprecipitations.

The LOABeads IP30 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.

2. Antibody affinity to protein A

Table 1. Binding strengths for protein A

Species	Subclass	Affinity
Human	IgG_1	++
	IgG_2	++
	IgG_3	-
	IgG_4	++
	IgA	+
	IgD	+
	IgE	+
	IgM	+
Mouse	IgG_1	+
	IgG_{2a}	++
	IgG_{2b}	++
	IgG_3	+
	IgM	+/-
Rat	IgG	++
	IgG_1	+/-
	IgG_{2a}	+/-
	IgG_{2b}	+/-
	IgG_{2c}	+/-
	IgM	+/-

Species	Subclass	Affinity
Rabbit		++
Hamster		+
Guinea Pig		++
Bovine		+
Horse		+
Sheep		+/-
Goat		+/-
Pig		++
Chicken	IgY	-

Antibody fragments		Affinity
Human	Fab	+
	F(ab')2	+
	scFv	+
	Fc	+
	κ	-
	λ	-

++ 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 IP30

Matrix	Super-paramagnetic agarose
Product	LOABeads IP30, 10% bead suspension
Ligand	Recombinant protein A
Particle size	45–165 µm
Binding capacity[1]	30 mg IgG/ml settled beads
Binding conditions	pH 6–8
Elution conditions[2]	SDS-PAGE Loading Buffer 60 mM citrate pH 3.0 100 mM glycine, pH 2.8
Storage	+2 to +8°C in PBS with 20% ethanol.
Stability[3]	18 months
Protein A ligand leakage[4]	10–100 ng/mg IgG (10–100 ppm)
Reusability	Not for reuse

1 Binding capacity was determined by incubating 50 µl LOABeads IP30 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. Material supplied

- LOABeads IP30 is supplied as a 10% bead suspension in PBS with 20% ethanol. 10 ml 10% bead suspension contains 1 ml beads.

5. Additional materials needed

- Washing buffer – For washing of beads, use the same buffer as used for obtaining the specific protein lysate. For instance, if the cells or tissue were homogenized with TBS + Triton X-100, use the same buffer to equilibrate the beads and to wash the beads after target protein adsorption. TBS (50 mM Tris-HCl pH 7.5, 150 mM NaCl).
- Elution buffer – For release of the antigen-antibody complex from the agarose resin, normally use SDS-PAGE loading buffer, supplemented with, e.g., 65 mM DTT. Other conditions might apply, depending on downstream application. For instance, DTT can be omitted if non-reduced separation on SDS-PAGE is desired. Other release buffers, includes 60 mM citrate, pH 3.0, or 100 mM glycine, pH 2.8.
- Mixer – It is important to obtain a continuous gentle mix to ensure that the beads are constantly in suspension with the sample. An end-over-end mixer is a good choice, but a benchtop shaker or a rocking table can be used for larger tubes. Manual inversion of the vial can also be applied.
- Magnetic separator – LOABeads MagSep5 (Product No. 2100) is suitable for separations in 0.5–5 ml volumes. For separation of volumes larger than 5 ml, use LOABeads MagSep50 (Product No. 2200) for volumes up to 50 ml.
- 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

- LOABeads MagSep5 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 MagSep50 separator. 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 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.

Adding beads to sample

- Equilibrate the beads in TBS or similar buffer as used for lysing the tissue or cells. Separate the beads against the wall and remove the supernatant. Resuspend the beads in a small amount of sample, transfer everything back to the sample tube, and continue with the adsorption phase.

Incubation

- Incubations should be performed with continuous mixing, usually with an end-over-end apparatus, or for larger tubes, a bench-top shaker or a rocking table.
- LOABeads IP30 can be used both at room temperature, as well as in a cold room; with the latter usually requiring longer incubation times.

7. Product operation

Intended use

- This product is intended for immunoprecipitations and similar applications where an IgG antibody is immobilized on protein A beads. For applications where the antibody needs to remain attached to the bead during the complete process, instead couple the antibody covalently to a suitable bead, such as the activated LOABeads AffiActive10.

Immunoprecipitation

Three strategies

An immunoprecipitation can be performed in three ways: (i) the antibody can be added to the sample to bind to its target, whereafter the beads are added to capture the antibody-antigen complex, (ii) the antibody is pre-bound to the beads, whereafter the antibody-bead complex is added to the sample to capture the target protein, and (iii) the antibody is covalently coupled directly to the bead, to minimize the presence of antibody in downstream analysis. The latter, however, is instead performed with product LOABeads AffiActive10.

Amount of antibody and beads

- A good starting point is 2 µg of antibody per immunoprecipitation, but this might need to be optimized depending on the specific antibody, type of sample, the concentration of the targeted molecule, and volume of sample. A range from 0.5 to 8 µg antibody might need to be tested.
- The amount of magnetic agarose bead used can vary from 2 to 20 µl.
- Smaller amounts of beads require longer incubation times, but elution can be performed with a lower amount of buffer.
- The amount of protein lysate to use depends on the expression levels of the specific protein and this needs to be empirically determined. For instance, a house holding protein might be expressed at much higher levels than a transcription factor. Also, the purpose of the immunoprecipitation matters. To obtain a workable Western blot image with high sensitive HRP-substrates might require a smaller amount of protein lysate, while being able to visually see the target protein on a silver stained gel for mass spectrometry identification, normally need a significantly larger amount of starting material.

Pre-adsorbing antibody to the protein A beads

- When using the same antibody with many samples, a stock of pre-coupled beads can be prepared. Shortly, aliquot 100 µl beads into a microtube, separate and discard the supernatant. Wash once or twice with TBS. Resuspend beads in 380 µl TBS and add, e.g., 20 µg antibody. Mix gently for 1 to 3 hours, separate and wash the beads at least two times with 0.9 ml TBS. Resuspends beads in 0.9 ml TBS or protein lysate buffer, to obtain a 10% beads suspension. Use, for instance, 5 µl beads per immunoprecipitations.

Elution conditions

- The type of analysis to be performed, affects elution conditions. If a non-reduced SDS-PAGE is to be performed, DTT is omitted from the elution buffer. For other applications, beside gel electrophoresis, elution can be performed by low pH, such as 60 mM citrate pH 3.0 or 100 mM glycine pH 2.8.
- During the above elution conditions, the antibody as well as its antigen will be released. In some cases, this becomes an issue since the release IgG antibody might mask the analysis of the actual targeted protein. In these cases, the antibody can be coupled covalently directly onto the agarose resin. For this, use LOABeads AffiActive10 where proteins and peptides can be coupled covalently through primary amines and free thiols.

Unspecific binding – background

- LOABeads IP30 is based on agarose resin, a matrix that thanks to its hydrophilic nature provide a low level of unspecific binding.
- Background issues during an immunoprecipitation do exist, however. Depending on the sensitivity of the downstream analysis method, all beads used for immunoprecipitation can show unspecific binding as background. Different brands of beads can differ in the degree of crosslinking, type of synthetic bead, and the amount of protein A, and therefore variation in background can be detected when comparing beads of different brands.
- In an immunoprecipitation followed by a Western blot, background can strongly correlate to the specific antibody used for the pulldown, as well as the primary antibody used for detection of target protein on the Western blot membrane. The source of the protein lysate (type of tissue, cell line, or species) can also contribute to differences seen on background in a Western blot image.

- In a Western blot, unspecific bands usually do not matter, as long as they do not interfere with the target band of interest; the protein that the primary antibody is directed against.
- If unspecific bands in a Western blot image do interfere, there are several ways to work around this. Optimization of different parameters include for instance the type of lysis buffer for the tissue or cells and washing buffer. The ion content, as well as detergent, can be changed. The amount of antibody used in each individual pulldown can be decreased, the amount of primary antibody on the Western blot membrane can be optimized.

8. General protocol

Immunoprecipitation for SDS-PAGE

1. Add 2 µg of polyclonal or monoclonal antibody to a sample, usually 0.5 to 1 ml protein lysate.
2. Mix end-over-end for 30 min to 3 hours at room temperature or 3 hours to overnight in a cold-room.
3. Add 10 µl prewashed LOABeads IP30 to the sample
4. Mix for another 30 min to 2 hours.
5. Centrifuge briefly to get liquid inside the lid down in solution.
6. Separate beads using the magnet and withdraw the supernatant.
7. Wash at least 3 times with 0.5 ml protein lysate buffer or TBS.
8. Add 2x or 4x SDS-PAGE loading buffer and DTT, to obtain 1x and 65 mM, respectively, in a final volume of 20 to 40 µl.
9. Vortex gently.
10. Heat 3–5 min at 95°C
11. Vortex gently and let cool.
12. Spin down any moisture in the lid and vortex gently again.
13. Separate beads magnetically and load supernatant on SDS-PAGE

9. Disclaimer

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

LOABeads is a trademark of Lab on a Bead AB

Tween is a registered trademark of Croda Americas LLC

10.Contact and ordering information

Products	Quantity	Product no.
LOABeads IP30	0.5 ml beads	1401
LOABeads IP30	1 ml beads	1402
LOABeads IP30	5 ml beads	1403

Related products	Product no.
LOABeads AffiActive10	1300
LOABeads MagSep5	2100
LOABeads MagSep50	2200

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