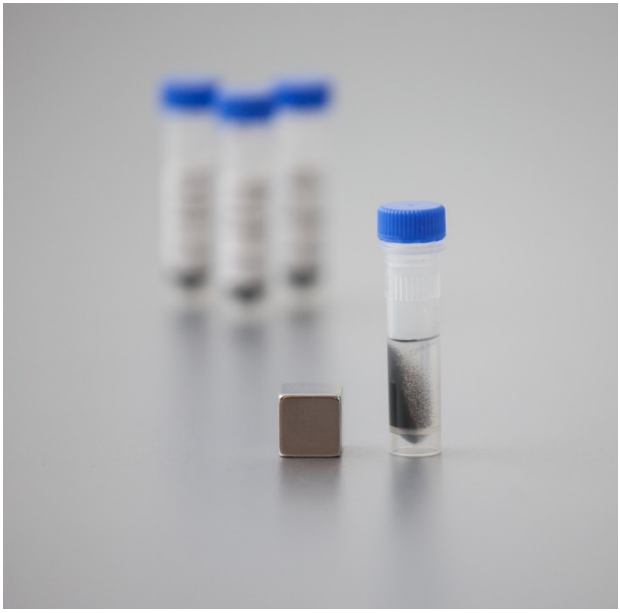
MAGIC BEADS

MAGic™Beads ACT

Magnetized bioseparation,
custom coupling of 10-30 mg protein/ml

User Instruction



MAGic Bioprocessing AB

Revision date 2024-02

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Please read through this manual carefully before using MAGic™Beads ACT.

Intended use

This product is intended for covalent coupling of proteins and peptides for use in affinity applications such as purification and immunoprecipitation.
For research use only.

1. General information

MAGic™Beads ACT consists of super-paramagnetic agarose beads, which are functionalized for covalent coupling of molecules with primary amino and thiol groups, such as proteins and peptides. Subsequently, target molecules can be affinity purified using magnetic separation technology.

The MAGic™Beads ACT 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.

Our patented coupling technology allows rapid covalent linking of ligands to the beads under mild conditions in water-based media. The beads couple 10-30 mg IgG per ml settled beads and 5–7.5 mg protein A per ml settled beads. The bond is very stable (6 ppm leakage of protein A).

In downstream applications, the quantity of beads can easily be scaled up or down to match target protein concentration and sample volumes.
In addition to applications in microcentrifuge tubes, our series of MAGic™Accio separators (purchased separately) enables handling of sample volumes up to 2000 ml.

2. Product data

Coupling to	Primary amino and thiol groups
Matrix	Super-paramagnetic 4% agarose
Particle size	45–165 µm
Product form	10% bead suspension in PBS with 20% ethanol
Coupling capacity[1]	5–10 mg IgG/ml settled beads
Coupling buffer	PBS with 0.1% Tween® 20
Storage	+2 to +8°C in PBS with 20% ethanol
Shelf life[2]	24 months

[1] Coupling capacity was determined by incubating 0.1 ml MAGic™Beads ACT with rabbit IgG (1 mg/ml in 2 ml PBS) for 60 minutes at room temperature.
[2] Data of product stability is continuously updated based on ongoing stability studies.

3. Material supplied

- MAGic™Beads ACT supplied as a 10% bead suspension in PBS with 20% ethanol. 1 ml 10% bead suspension contains 100 µl beads.
- Activation buffer
- Blocking buffer (50% v/v ethanolamine)

4. Additional materials needed

- **Coupling/Washing buffer** – For coupling of proteins to beads and for washing, PBS (137 mM NaCl, 2.7 mM KCl, 10 mM phosphate, pH 7.4) with 0.1% Tween® 20.
- **Storage buffer** – Store beads in PBS with 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** – MAGic™Accio LAB (Product No. 2000, 2100) is suitable for separations in 0.5–5 ml volumes. For separation from volumes larger than 5 ml, use MAGic™Accio PILOT 50 (Product No. 2200) for volumes up to 50 ml, MAGic™Accio PILOT 500 (Product No. 2300) for volumes up to 500 ml, or MAGic™Accio PILOT 2000 (Product No. 2400) for volumes up to 2000 ml (Section 11).
- Additional vials/tubes, pipettes, and pipette tips.

5. 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 MAGic™Accio LAB 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 MAGic™Accio PILOT 50 separator. Use the MAGic™Accio PILOT 500 separator for volumes up to 500 ml. For volume large and up to 2000 ml use the MAGic™Accio PILOT 2000 (Section 11). 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 can be performed by manual mixing/inversion of the test tube or bottle.
- Coupling can be performed at room temperature.

6. Product operation

Intended use

- This product is intended for covalent coupling of proteins and peptides for use in affinity applications such as purification and immunoprecipitation.
- The product should not be used for applications in solutions containing high levels of free thiols.

Coupling

- MAGic™Beads ACT link to proteins and peptides through primary amino and/or thiol groups. It is recommended to use PBS (137 mM NaCl, 2.7 mM KCl, 10 mM phosphate, pH 7.4) containing 0.1% Tween® 20 as the coupling and washing buffer.
- The coupling capacity of the beads is generally 10-20 mg protein or 2–4 mg peptide per ml of settled beads, reached within 1 hour with ~90% yield.
- To maximize coupling through the sidechain of a cysteine residue in a peptide, the peptide should first be exposed to a reducing environment through, e.g., DTT, and thereafter gel-filtrated to remove the DTT.

Washing

- To wash the beads during the coupling procedure, use PBS containing 0.1% Tween® 20.

Storage

- The MAGic™Beads ACT particles should be stored as a 10% bead suspension at +2 to +8°C in PBS containing 20% ethanol.

7. General protocol to couple 100 µl beads with protein

The coupling protocol can be scaled up proportionally if larger amounts of beads are needed.

Bead preparation

1. Dispense 1 ml of 10% bead suspension (100 µl beads) in a test tube.
2. Remove the storage solution by magnetic separation.
3. Resuspend beads in 1 ml of coupling buffer.
4. Remove the liquid by magnetic separation.
5. Resuspend beads in 1 ml of coupling buffer.

Activation

6. Add 50 µl of activation buffer and incubate for 15 min with continuous end-over-end mixing.
7. Remove activation solution from beads by magnetic separation.
8. Resuspend beads in 1 ml of coupling buffer.
9. Remove the liquid.

Coupling

10. Prepare the protein (1 mg) to be coupled, as a solution with a concentration of 1 mg/ml in coupling buffer.

Note: It is important that the protein does not have any amino-containing impurities or contain ammonium sulfate, as these would also bind to the reactive structures. The product is not suitable for applications in solutions containing high levels of thiols.

11. Add the protein solution to beads and allow coupling for 1 hour at room temperature with continuous end-over-end mixing.
12. Coupling efficiency of proteins and peptides could be determined by measuring A_{280} of the ligand solution before and after coupling to beads. For peptides and proteins, with poor absorbance at 280 nm, the absorbance of the peptide back bone bonds can be measured at, e.g., 214 nm.

Washing

13. Remove coupling solution from beads by magnetic separation.
14. Wash out unbound ligand with 1 ml of coupling buffer.
15. Repeat washing step twice.

Blocking

16. Remaining reactive groups on beads are blocked with blocking buffer.
17. Add 1 ml PBS to the settled beads + 80 µl Blocking buffer.
18. Allow blocking for 45 min at room temperature with continuous mixing.
19. Wash with 1 ml of coupling buffer and repeat washing step twice.
20. Resuspend beads in 900 µl of storage buffer, to obtain a 10% bead suspension, unless beads are to be used directly in downstream applications.

8. Using coupled beads

MAGic™Beads ACT are ready for use in downstream applications when the beads have been coupled with a ligand. Below, a general protocol for an immuno-affinity purification and an immunoprecipitation using MAGic™Beads ACT magnetic particles can be found. Other examples of different applications can be found in application notes on our webpage.

Purification of anti-rabbit IgG using MAGic™Beads-rabbit–IgG

Coupling of rabbit IgG to beads

The coupling of rabbit IgG to the MAGic™Beads ACT particles is performed as described in the coupling protocol in Section 7.

Purification of anti-rabbit IgG antibody from 1 ml goat antiserum

Bead preparation

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

Sample application

6. Add 1 ml goat serum, containing antibodies raised against rabbit IgG, to the beads.

7. Incubate with continuous mixing using an end-over-end mixer for 30–60 min.
8. Remove the liquid.

Washing

9. Add 1 ml PBS, resuspend the beads, and mix for 30 sec by manual inversion.
10. Remove the liquid.
11. Perform steps 9 and 10 a total of three times.

Elution

12. Add 500 µl of elution buffer (100 mM citrate, pH 2.8).
13. Resuspend the beads and mix for 1 min by manual inversion.
14. Remove and collect the elution fraction. Generally, 85–90% of bound antibody is found in the first elution fraction.
15. Repeat elution step if necessary.
16. For neutralization of eluted antibodies, add, e.g., 1/10 fraction volume of 2 M Tris-HCl, pH 9.0, to each elution fraction.
17. To regenerate the beads, wash up to four times with 1 ml elution buffer and twice with 1 ml binding buffer. Resuspend in 0.9 ml of storage buffer.

Immunoprecipitation using MAGic™Beads ACT

Coupling of IgG antibody to beads

Monoclonal or polyclonal antibodies are immobilized covalently to the MAGic™Beads ACT particles, as described in the coupling protocol in Section 7. For immunoprecipitation, 40 µg antibody (see below) is coupled per 100 µl beads.

Immunoprecipitation for SDS-PAGE

1. If using stored beads, pre-wash beads and resuspend in protein lysate buffer, TBS or PBS as a 10% suspension.
2. Add 5 µl (50 µl bead suspension) of antibody-coated bead per aliquoted sample, usually 0.5 to 5 ml protein lysate.
3. Mix end-over-end for 1 to 3 hours at room temperature or 3 hours to overnight in a cold-room.
4. Spin microcentrifuge tubes briefly to move liquid inside the lid down in solution.
5. Separate beads using the magnet and withdraw the supernatant.
6. Wash at least 3 times with 0.9 ml protein lysate buffer or TBS.
7. Add a suitable amount of SDS-PAGE loading buffer containing SDS to the beads. Vortex gently.
8. Heat 3 min at 95°C
9. Vortex gently and let cool slightly.
10. Separate beads and transfer the supernatant to a new tube.

11. Optional, add DTT to a final concentration of, e.g., 65 mM, heat again for 1-2 min at 95°C, and let cool to room temperature
12. Separate sample on SDS-PAGE.

Amount of antibody

The amount of antibody used in an immunoprecipitation varies and differ most significantly from when performing a classic preparative affinity purification. A standard amount of IgG antibody used for immunoprecipitation followed by separation on SDS-PAGE, and subsequent Western blot analysis, typically starts at 2 µg antibody per 5 µl beads, but anywhere from 0.5 to 8 µg might be used. Therefore, covalently coupling 40 µg of antibody per 100 µl of MAGic™Beads ACT is a good start, but the specific downstream analysis application might require a different amount of antibody per 100 µl beads.

Amount of beads

Normally, 5 µl magnetic agarose beads are used per immunoprecipitation, but a range from 2 to 20 µl might can also be used.

Reducing agents

The proprietary coupling chemistry used in MAGic™Beads ACT, which enables coupling of ligands at physiological conditions, produces stable bonds between the resin matrix and ligand. However, during elution of the target protein, DTT or other strong reducing agents should not be used, as they might break the covalent bond between the ligand and the bead. If reduced proteins are needed for downstream analysis, reduction should be performed on the eluted proteins in a separate tube. In addition, purification of secreted proteins from cell culture media containing substantial amounts of thiols, should be avoided.

9. 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 micro centrifuge.

10. Disclaimer

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

11. Ordering information

Products	Quantity	Product No.
MAGic™Beads ACT	1 ml beads	1301
MAGic™Beads ACT	5 ml beads	1302
MAGic™Beads ACT	25 ml beads	1303
MAGic™Beads ACT	50 ml beads	1304
MAGic™Beads ACT	250 ml beads	1305
MAGic™Beads ACT	1 l beads	1306

Related products	Product No.
MAGic™Beads custom	1000
MAGic™Beads SerA	1200
MAGic™Beads mAb	1500
MAGic™Accio LAB rack	2000
MAGic™Accio LAB cube	2100
MAGic™Accio PILOT 50	2200
MAGic™Accio PILOT 500	2300
MAGic™Accio PILOT 2000	2400

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