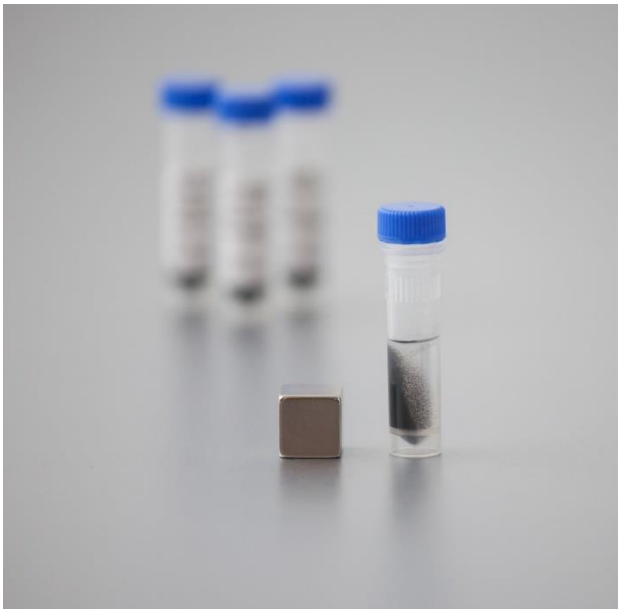
CD Creative Diagnostics®

Product no WHM-L087

Absolute Mag™ Amine

Product Manual



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Please read through this manual carefully before using Absolute Mag™ Amine.

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

Absolute Mag™ Amine 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 Absolute Mag™ Amine 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 linking of ligands to the beads under mild conditions in water-based media. The beads couple 0.5–1 mg of IgG per ml bead suspension (5–10 mg per ml settled beads) and 0.5–0.75 mg protein A per ml bead suspension (5–7.5 mg 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 Absolute Mag™ MagSep separators (purchased separately) enables handling of sample volumes up to 500 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]	0.5–1 mg IgG/ml 10% bead suspension 5–10 mg IgG/ml settled beads
Coupling buffer	PBS with 0.1% Tween® 20
Storage	+2 to +8 ℃ in PBS with 20% ethanol
Shelf life[2]	6 months

[1] Coupling capacity was determined by incubating 1 ml 10% Absolute Mag™ Amine with rabbit IgG (1 mg/ml in 1 ml PBS) for 60 minutes at room temperature.
[2] Data of product stability is continuously updated based on ongoing stability studies.

3. Material supplied

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

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 phospate, pH 7.4) with 0.1% Tween® 20.
- **Blocking buffer** – To block remaining reactive groups on the beads use ethanolamine (50 vol% in PBS).
- **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** – For separation from volumes larger than 5 ml, use Absolute Mag™ MagSep 15/50 (Product No. 3001) for volumes up to 50 ml, or Absolute Mag™ MagSep 500 (Product No. 4001) for volumes up to 500 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 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 Absolute Mag™ MagSep 15/50 separator. Use the Absolute Mag™ MagSep 500 separator for volumes up to 500 ml (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

- Absolute Mag™ Amine 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 phospate, pH 7.4) containing 0.1% Tween® 20 as the coupling and washing buffer.
- The coupling capacity of the beads is generally 0.5–1 mg protein or 0.2–0.4 mg peptide per ml 10% bead suspension, 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 gelfiltrated to remove the DTT.

Washing

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

Storage

- The Absolute Mag™ Amine particles should be stored as a 10% bead suspension at +2 to +8 ℃ in PBS containing 20% ethanol.

7. General protocol to couple 100 µl beads with protein

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 intended 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 230 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 ethanolamine.
17. Add 80 µl ethanolamine solution (50 vol% in PBS) to the beads.
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

Absolute Mag™ Amine 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 using Absolute Mag™ Amine 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 Absolute Mag™ rabbit IgG

Coupling of rabbit IgG to beads

The coupling of rabbit IgG to the Absolute Mag™ Amine particles was 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 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 (60 mM citrate, pH 3.0).
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.

9. Practical notes

- Instead of using a magnetic separator in order to separate beads from solution in the washing steps, beads can also be washed on a sintered filter funnel Por 2 or 3.
- 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.