

Coupling AbraMag[®] Amine Magnetic Beads

Materials:

1. AbraMag Amine (NH₃) Magnetic Beads (PN 544080 & PN 544081)
2. Coupling Buffer: 0.1M MES pH 6.0
3. Glutaraldehyde solution, 25% in H₂O, Grade I, Sigma cat# G5882
4. Quenching Buffer (blocking buffer): 1M Glycine pH 8.0
5. 1x PBS (phosphate buffered saline)
6. 15 ml or 50 ml conical or centrifuge vials
7. Rotator or shaker
8. Vortexer (optional)
9. Magnetic Separator (e.g. <http://www.abraxiskits.com/products/magnetic-separators/>)
10. Purified protein or antibody in Coupling Buffer at a concentration between 0.1 to 2.0 mg/ml. If already in a different solution that contains primary amines (e.g. glycine or tris), it must be buffer-exchanged by dialysis or desalting.
11. Storage buffer (e.g. PBS w/ 0.1% BSA and preservative such as sodium azide)

Notes and Precautions:

- This product is for *in vitro* diagnostics use only.
- Do not freeze reagents. Store at 2 – 8°C.
- Do not freeze, dry, or centrifuge magnetic beads as this may result in irreversible aggregation and loss of binding activity. Similarly, do not allow to dry during procedure.
- Prior to use, ensure that the product has not expired by verifying that the date of use is prior to the expiration date on the label.
- Ensure that reagent bottle caps are tight after each use to prevent evaporation.
- Reagents contain 0.05-0.1% sodium azide as a preservative. Sodium azide may react with lead or copper plumbing to produce metal azides, which might cause explosion. To prevent azide accumulation in plumbing, flush with copious amounts of water immediately after disposal.

Procedure:

Calculate approximate ratio and amounts of beads and protein/antibody to couple.

Beads: for example, 1 ml beads at 2.5% solids = 25 mg.

Typically, start with 0.5-5.0 mg of purified protein per 25 mg (1 ml) beads.

1. Add 1 ml beads to 9 ml of Coupling Buffer, vortex to mix well. Place on magnetic separator and separate for 10 minutes, then draw out or decant supernatant while tube is still on magnetic separator.
2. Add 10 ml of Coupling Buffer to beads, vortex to mix well. Place on magnetic separator and separate for 10 minutes, then draw out or decant supernatant while tube is still on magnetic separator.
3. Repeat step 2 once.
4. Activate: Add glutaraldehyde to final 5% concentration. For example, resuspend 1 ml of beads in 2.5 ml of Coupling Buffer, and then add 0.5 mL of 25% glutaraldehyde.
5. Rotate or shake vigorously for 3 hours at room temperature. Separate for 10 minutes, then draw out or decant supernatant.
6. Add 10 ml Coupling Buffer to wash. Separate for 10 minutes, then draw out or decant.
7. Repeat step 6 twice.
8. Add 0.5-5.0 mg (as calculated above) protein/antibody in Coupling Buffer.
9. Vortex, then rotate or shake overnight at room temperature.
10. Separate for 10 minutes, then draw out or decant supernatant.

11. Add 10 ml of Quenching Buffer, vortex, rotate for 30 minutes at room temperature. Separate for 10 minutes, then draw out or decant.
12. Wash: add 10 mL of 1x PBS, vortex, and separate for 10 minutes, then draw out or decant.
13. Repeat step 12 three more times.
14. Add 2.5 ml of Storage Buffer for 10 mg/ml beads. *Caution:* we have not yet tested the storage stability in this buffer.

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