Coupling AbraMag® Carboxyl Magnetic Beads via NHS-Activation

Materials:

1. AbraMag® Carboxyl (COOH) Magnetic Beads (PN 544085 & PN 544086)
2. Coupling Buffer: 0.1M MES pH 6.0
3. EDAC (1-Ethyl-3-(3-dimethylaminopropyl) Carbodiimide Hydrochloride) (e.g. Sigma #E1769)
4. NHS (N-hydroxysuccinimide) (e.g. Sigma # 130672)
5. Quenching Buffer (blocking buffer): 50 mM Ethanolamine pH 8.0 + 0.1% BSA
6. 1x PBS (phosphate buffered saline)
7. 15 ml or 50 ml conical or centrifuge vials
8. Rotator
9. Vortex (optional)
10. Magnetic Separator
11. 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.
12. 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 EDAC to a concentration of 10 mg/ml and NHS to 3 mg/ml. For example, resuspend 1 ml of beads in 2.5 ml of Coupling Buffer, then add 25 mg EDAC and 7.5 mg NHS and mix well. Alternatively, EDAC and NHS can be added to the Coupling Buffer, but the solution must be used immediately to resuspend the beads, or activation efficiency will be reduced.
5. Rotate for 15 minutes 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 once.
8. Add 0.5-5.0 mg (as calculated above) protein/antibody in Coupling Buffer.
9. Vortex, then rotate for 3 hours or 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, 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.

For ordering or technical assistance contact:

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