Carboxyl Magnetic Beads

Catalog No. MBFB-03001, MBFB-03002

【Introduction】

Carboxyl Magnetic Beads are nano-superparamagnetic beads coated with carboxylic acid functional groups, which can be activated (e.g. using EDC and NHS) and then reacts with amines on nucleic acid, protein, antibodies or other molecules to form stable amide linkages. After that, the beads can be separated from the solution using magnet for downstream experiments, such as enzymatic reactions or immunopurification of other large molecules.

【Product Specifications】

- **Diameter:** 500nm
- **pH stability:** pH 4-12
- **30min sedimentation rate:** <0.1%
- **Magnetic response rate:** >30emu/g
- **Solvent:** 20% ethanol
- **Binding capacity:** 10-100μg protein per mg carboxylated magnetic beads

【Product Content】

Catalog No.	*Conc. (mg/ml)*	*Volume (ml)*	*Amount of Beads (mg)*
MBFB-03001	50	2	100
MBFB-03002	50	20	1000

【Coupling Protocol】

The following protocol provides general guidelines for coupling of proteins or antibodies to 200µl of Carboxylated Magnetic Beads. The protocol is scalable. Optimization of the coupling conditions (protein concentration, coupling buffer, pH, and incubation time) for the ligand of interest is recommended. Other general activation protocols are also applicable.

A. Notes

1. Amine-containing buffers (e.g. Tris and glycine) inhibit coupling of protein to the magnetic beads. If protein is dissolved in a buffer containing primary amine, remove the buffer using dialysis or desalting.
2. The optimal protein concentration should be optimized. Too low a protein concentration may result in bead crosslinking. For coupling of expensive antibodies that may not be available in enough quantity to reach the desired concentrations, another protein (i.e. BSA) may be added to take up remaining reactive sites.

B. Additional materials required

1. Coupling Buffer: 50mM MES, pH 6.0
2. NHS and EDC
3. Blocking Buffer: 100mMTris-HCl, pH 7.0
4. Protein Solution: dissolve protein in 5ml of Coupling Buffer. Protein concentration is typically 1-10mg/ml
5. A magnetic stand

C. Two-step EDC/NHS activation and coupling procedure

Step 1: Activation (using EDC/NHS activation as an example)

1. Gently mix the magnetic beads thoroughly before use by shaking.
2. Place 20µl of magnetic beads (1mg) into a microcentrifuge tube.
3. Place the tube into a magnetic stand, collect the beads and discard the supernatant.
4. Wash the beads three times with Coupling Buffer (1ml each time) by magnetic separation, and re-suspend the beads in 2ml of Coupling Buffer.
5. Add 5mg of EDC and 5mg of NHS. Mix to dissolve. To facilitate faster dissolution, EDC and NHS may be dissolved immediately before use as a concentrated stock solution in Coupling Buffer and then an aliquot of this solution is added to the bead suspension to obtain the correct final concentration.

6. React for 15min at room temperature.
7. Quickly wash the beads twice with Coupling Buffer (1ml each time) by magnetic separation, and then re-suspend the beads in 5ml of Coupling Buffer.

Step 2. Coupling with protein

1. Add 5ml of the protein solution to 5ml of the bead suspension. Mix thoroughly and incubate for 2-4hrs at room temperature or overnight at 4°C on a rotator.
2. Collect the beads with a magnet and save the supernatant for analysis if needed.
3. Add 5ml Blocking Buffer. Mix thoroughly and incubate for 2-4hrs at room temperature or overnight at 4°C on a rotator.
4. Wash the protein-coupled beads twice with Blocking Buffer (1ml each time) by magnetic separation.
5. Suspend the beads with a desired volume of Coupling Buffer or in a buffer compatible with the attached protein. Store at 4ºC until ready for use.

【Storage】

Stored at 2-8°C, 2 years.

【Manufacturer】

Avanbio, ABF-03001, ABF-03002

【Troubleshooting】

A. Protein was not soluble in coupling buffer
   Protein may be hydrophobic. DMSO (up to 20%) can be added to coupling buffer before dissolving the protein.
B. Coupling efficiency was low
   Buffer containing primary amine was not completely removed. Please desalt or dialyze protein sample before coupling to completely remove primary amine such as Tris and glycine.