Buffer Kit for Coupling of Carboxyl Bead

DESCRIPTION

Magnetic beads are commonly used as coupling carriers. This kit provides the buffer needed for the coupling of carboxyl and amino groups, such as carboxyl microbeads coupled antibodies, amino modified DNA, etc. The carboxyl magnetic bead coupling kit is suitable for covalently coupling biological ligands such as proteins and antibodies to the surface of microspheres for further protein purification and immunoassay.

PRODUCT INFORMATION

Buffer	Size	Compositon
Coupling Buffer	50 ml	50 mM MES, pH 6.0, 0.01% Triton X-100
Coupling Agent	50mg×1	EDC； 50 mg/ml × 1ml （add 1ml Coupling Buffer before use）
	50mg×1	Sulfo-NHS； 50 mg/ml × 1ml （add 1ml Coupling Buffer before use）
Quench Buffer	50 ml	TBS (25 mM Tris-Cl, 130 mM NaCl, 2.7 mM KCl), pH 8, 0.01% Triton X-100
Storage Buffer	10 ml	TBS or PBS containing 0.01% Triton X-100 or 0.01% Tween 20, 0.05%NaN_3

PROTOCOL

This protocol increases the efficiency of EDC-mediated reactions by adding sulfo-NHS that stabilizes the amine-reactive intermediate. The amount of coupling objects involved in this scheme (proteins, amino-modified nucleic acids, and other compounds containing primary amino groups) can be further optimized as needed to achieve the optimal coupling rate.

1. Make sure the protein or ligand is in amine-free coupling buffer. 50-400 μg of protein in 100 μL of coupling buffer is required for conjugation.

2. Vortex to resuspend the Carboxy Magnetic Beads. Pipette 100 μL of magnetic beads into a 1.5 mL EP tube, magnetically separate and discard the supernatant.

3. Add 200 μL of coupling buffer. Vortex vigorously for 20 seconds, magnetically separate and discard the supernatant.

4. Follow step 3 to wash the carboxyl magnetic beads two more times.

5. Prepare EDC (50 mg/mL) with coupling buffer before use. Sulfo-NHS (50 mg/mL) was prepared with coupling buffer before use.

6. Add 60 μL of coupling buffer, 20 μL of freshly prepared EDC solution, and 20 μL of freshly prepared Sulfo-NHS solution to the magnetic beads. Mix well.

7. Mix and incubate for 15 minutes at room temperature. Magnetically separate and discard the supernatant.

8. Wash the beads with 200 μL of coupling buffer. Vortex to mix, magnetically separate and discard the supernatant.

9. Add 100 μL of coupling buffer and 50 to 400 μg of protein or ligand. Vortex to mix. Incubate with continuous mixing for 0.5 to 4 hours at room temperature.

10. Put the test tube into the magnetic stand, magnetically separate and discard the supernatant. This supernatant contains unbound ligand and can be saved for analysis if the protocol needs to be optimized.

11. Add 250 μL of Quenching Buffer to the Carboxy Magnetic Beads. Vortex for 20 seconds, magnetically separate and discard the supernatant.

12. Add 500 μL of Quenching Buffer to the Carboxy Magnetic Beads. Incubate at room temperature for 30 to 60 minutes. Magnetically separate and remove the supernatant.

13. Add 250 μL of Quenching Buffer to the Carboxy Magnetic Beads. Vortex vigorously for 20 seconds, magnetically separate and discard the supernatant.

14. Remove the EP tube from the magnetic stand. Add 100 μL of storage buffer. Vortex to mix and store coupled beads at 2-8°C.

Note

Coupling Agent: It is recommended to prepare and use now, and the solution should not be stored for more than 1 month.

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