Coupling of proteins to PureCube Carboxy MagBeads

Overview

This protocol delineates a coupling procedure for proteins to PureCube Carboxy MagBeads using the cross-linking reagents EDC and NHS. Proteins are coupled covalently and can be used for different applications, e.g. purification of interaction partners.

Amounts given in this protocol are for 1 mL Carboxy MagBead suspension, which contains 250 µL magnetic beads. This reaction can be linearly scaled up or down using appropriate magnetic holders. Magnetic holders for a wide range of volumes are available e.g. from Sepmag (www.sepmag.eu).

Please contact us if you have questions or need assistance optimizing a protocol for your application (contact@www.cube-biotech.com); other protocols can also be found at www.cube-biotech/protocols.

Equipment

- ☐ Magnetic holder for microcentrifuge tubes (for separation of magnetic beads)
- ☐ Microcentrifuge tubes (2 mL)
- ☐ End-over-end mixer or thermomixer
- ☐ Spectrophotometer

Materials

- ☐ PureCube Carboxy MagBeads (1 mL, Cube Biotech #50201)
- ☐ Sodium dihydrogen phosphate
- ☐ Sodium chloride
- ☐ Sodium acetate trihydrate
- ☐ Sodium hydroxide (NaOH)
- ☐ Acetic acid
- ☐ Ethanol
- ☐ Ethanolamine
- ☐ N-(3-Dimethylaminopropyl)-N’-ethyl-carbodiimide hydrochloride (EDC)
- ☐ N-Hydroxysuccinimide (NHS)

Solutions and buffers

Phosphate buffer, pH 6.0, 250 mL

Component	Final concentration	Molecular weight (g/mol)	Stock concentration	Amount needed for buffer
NaH_2PO_4 dihydrate	25 mM	156.01	n.a.	975 mg
Instructions: Dissolve sodium phosphate in 200 mL water, adjust the pH to 6.0 with NaOH. Add water to a total volume of 250 mL.				

PBS Buffer, pH 7.2, 250 mL

Component	Final concentration	Molecular weight (g/mol)	Stock concentration	Amount needed for buffer
NaH_2PO_4 dihydrate	150 mM	156.01	n.a.	5.85 g
NaCl	100 mM	58.44	n.a.	1.463 g
Instructions: Dissolve components in 200 mL water, adjust the pH to 7.2 with NaOH. Add water to a total volume of 250 mL.				

Quenching Buffer, pH 7.4, 250 mL

Component	Final concentration	Molecular weight (g/mol)	Stock concentration	Amount needed for buffer
Ethanolamine	1 M	61.08	n.a.	15.27 g
Instructions: Dissolve component in 200 mL water, adjust the pH to 7.4 with HCl. Add water to a total volume of 250 mL.				

MagBead Storage Buffer, pH 6.5, 250 mL

Component	Final concentration	Molecular weight (g/mol)	Stock concentration	Amount needed for buffer
Sodium acetate trihydrate	20 mM	136.08	n.a.	674 mg
Ethanol	20 % (v/v)		100 % (v/v)	51 mL
Instructions: Dissolve sodium acetate in 150 mL water, adjust the pH to 6.5 with acetic acid. Add 48 mL water and 51 mL ethanol to yield a total volume of 250 mL.				

Procedure

1. Transfer 1 mL PureCube Carboxy MagBeads into a 2mL microcentrifuge tube.
2. Place the tube on a magnetic stand and allow the beads to separate. Remove the supernatant. Resuspend the magnetic beads with 750 µL Phosphate Buffer.
3. Dissolve 63 mg NHS in 250 µL Phosphate Buffer, add it to the agarose suspension, and mix by vortexing.
4. Dissolve 63 mg EDC in 250 µL Phosphate Buffer, add it to the agarose suspension, and mix by vortexing.
5. Incubate at room temperature for 1 h on an end-over-end shaker or in a thermoshaker.
6. Prepare a solution of 625 µL PBS containing the protein to be coupled to the MagBeads. The exact protein amount needs to be optimized, and 1 to 3 mg protein is a good starting point.
7. Add the protein solution to the MagBeads and mix by vortexing.
8. Place the tube with the magbeads on a magnetic stand and allow the beads to separate. Remove the supernatant.
9. Wash the beads once with 1 ml PBS. Allow the beads to separate and remove the supernatant. **Important: Once PBS is added, work quickly to avoid hydrolysis of the NHS groups.**
10. Depending on the temperature stability of the protein, incubate at room temperature or 4°C for 2 h on an end-over-end shaker or thermoshaker.
11. Place the tube on a magnetic stand and allow the beads to separate. Remove the supernatant and analyze the supernatant in a spectrophotometer. Record absorption at 280 nm to monitor coupling efficiency.
12. Add 1.5 mL double distilled water to the MagBeads, mix by vortexing, and separate on a magnetic stand. Remove the supernatant.
13. Repeat step 10 five times.
14. Add 1.2 mL Quenching Buffer and incubate again for 1 h at room temperature or for 4 hours at 4°C.
15. Wash four times with 1.5 mL PBS each, and twice with 1.5 mL double distilled water each.
16. Resuspend the coupled MagBeads in 1 mL MagBead Storage buffer, yielding a 25% suspension. Store at 4°C.

Tip: The coupling reaction can be linearly scaled up and down, by increasing or decreasing the amounts of buffers and solutions described in this protocol.

Note: NHS and EDC should always be prepared fresh. Equilibrate the two chemicals to room temperature before weighing, and store the powders under protective gas (nitrogen). Add the two chemicals immediately one after another to prevent hydrolysis of the NHS-activated matrix.

Tip: When coupling a particular protein for the first time, try 3-5 different protein concentrations to make sure you are offering enough protein in the reaction but not wasting any protein.

Tip: Monitoring absorbance at 280 nm tells you about the coupling efficiency of the protein (compare A280 of the original protein solution to the supernatant in step 6 to determine % coupling). It also helps you identify the optimal amount of protein required for efficient coupling.

Note: The quenching step ensures that no free NHS groups are left on the MagBeads that might interfere with subsequent assays.

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