Coupling of proteins to PureCube NHS-Activated MagBeads

Overview

This protocol delineates a coupling procedure for proteins to PureCube NHS-Activated MagBeads. 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 NHS-Activated 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 NHS-Activated MagBeads (1 mL, Cube Biotech #50401)
- ☐ Sodium dihydrogen phosphate
- ☐ Sodium chloride
- ☐ Sodium acetate trihydrate
- ☐ Sodium hydroxide (NaOH)
- ☐ Acetic acid
- ☐ Ethanol
- ☐ Ethanolamine

Solutions and buffers

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 NHS-Activated MagBeads into a 2mL microcentrifuge tube.
2. Place the tube on a magnetic stand and allow the beads to separate. Remove the supernatant.
3. Wash the beads once with 1 ml PBS. Allow the beads to separate and remove the supernatant.
4. Prepare a solution of 625 µL PBS containg 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.
5. Add the protein solution to the MagBeads and mix by vortexing.
6. 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.
7. 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.
8. Add 1.5 mL PBS buffer to the MagBeads, mix by vortexing, and separate on a magnetic stand. Remove the supernatant.
9. Repeat step 7.
10. Wash four times with 1.5 mL double distilled water each.
11. Add 1.2 mL Quenching Buffer and incubate again for 1 h at room temperature or for 4 hours at 4°C.
12. Wash four times with 1.5 mL PBS each, and twice with 1.5 mL double distilled water each.
13. 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.

Important: Once PBS is added, work quickly to avoid hydrolysis of the NHS groups.

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.