

Coupling of proteins to PureCube Maleimide-Activated MagBeads

Overview

This protocol delineates a coupling procedure for proteins to PureCube Maleimide-Activated MagBeads. Proteins are coupled covalently and can be used for different applications, e.g. purification of interaction partners.

Maleimides react with free sulfhydryl groups at a pH 6.5-7.5, forming stable, covalent thioether bonds. At pH > 7.5, maleimide reacts with amine groups. Depending on the nature of the proteins to be coupled, two coupling buffers are described at pH 7.2 and 6.5, respectively. If you are unsure which buffer your protein is most stable in, it might make sense to try both buffers in a first small-scale experiment.

If cysteine groups in your protein are likely to be present in their oxidised form, add TCEP to the coupling buffer. This reducing agent should not affect the coupling reaction.

Amounts given in this protocol are for 1 mL Maleimide-Activated MagBead suspension, which contains 250 µL magnetic beads. This reaction can be linearly scaled up or down using appropriate magnetic holders.

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 (Cube Biotech #16941)
- Microcentrifuge tubes (2 mL)
- End-over-end mixer or thermomixer
- Spectrophotometer

Materials

- PureCube Maleimide-Activated MagBeads (1 mL, Cube Biotech #51201)
- Sodium dihydrogen phosphate
- Sodium chloride
- Sodium acetate trihydrate
- Sodium hydroxide (NaOH)
- Acetic acid
- Ethanol
- Optional: Sodium hydrogen carbonate
- Optional: Sodium azide
- Optional: Tris(2-chlorethyl)phosphate (TCEP)

Solutions and buffers

Coupling Buffer I (PBS) pH 7.2, 250 mL

| Component | Final concentration | Molecular weight (g/mol) | Stock concentration | Amount needed for buffer |
|---|---------------------|--------------------------|---------------------|--------------------------|
| NaH ₂ PO ₄ dihydrate | 100 mM | 156.01 | n.a. | 3.9 g |
| NaCl | 150 mM | 58.44 | n.a. | 2.195 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. For coupling of proteins with oxidised disulfide bonds, up to 25 mM TCEP can be added. | | | | |

Coupling Buffer II pH 6.5, 250 mL

| Component | Final concentration | Molecular weight (g/mol) | Stock concentration | Amount needed for buffer |
|--|---------------------|--------------------------|---------------------|--------------------------|
| Sodium acetate trihydrate | 100 mM | 136.08 | n.a. | 3.37 g |
| NaCl | 150 mM | 58.44 | n.a. | 2.195 g |
| Instructions: Dissolve components in 200 mL water, adjust the pH to 6.5 with acetic acid. Add water to a total volume of 250 mL. For coupling of proteins with oxidised disulfide bonds, up to 25 mM TCEP can be added. | | | | |

Storage Buffer I, 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. | | | | |

Storage Buffer II, pH 7.5, 250 mL

| Component | Final concentration | Molecular weight (g/mol) | Stock concentration | Amount needed for buffer |
|--|---------------------|--------------------------|---------------------|--------------------------|
| NaHCO ₃ | 100 mM | 84.01 | n.a. | 2.10 g |
| Sodium azide | 0.02 % (w/v) | 65.1 | 1 % (w/v) | 5 mL |
| Instructions: Dissolve sodium carbonate in 150 mL water, adjust the pH to 7.5. Add the sodium azide and fill up with water to a final volume of 250 mL. | | | | |

Procedure

1. Transfer 1 mL PureCube Maleimide-Activated MagBeads (25%) 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 Activated MagBeads three times with 1 ml coupling buffer I or II.
4. Resuspend 0.5-1.5 mg protein in 250 μ l coupling buffer I or II.
5. Add the protein solution to the magnetic beads and mix by vortexing.
6. Depending on the temperature stability of the protein, incubate at 20°C for 2 hours or at 4° C overnight on an end-over-end shaker or thermoshaker.
7. Remove the supernatant and analyze the supernatant in a spectrophotometer. Record absorption at 280 nm to monitor coupling efficiency.
8. Wash the magbeads three times with 1 ml coupling buffer and once with 1 ml double distilled water.
9. Resuspend the coupled MagBeads in 1 mL Storage buffer I or II, 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.

Tip: Choose coupling buffer I or II depending on the pH stability of your protein. Use the same coupling buffer throughout the procedure.

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.

