Activation, coupling and purification of proteins using PureCube Thiol-Activated MagBeads

Overview

NOTE: This protocol has been updated effective June 2016. Please ensure to follow this protocol and to discard previous versions.

This protocol delineates several procedures required for
A. Activation of PureCube Thiol-Activated MagBeads prior to use

B. Coupling of proteins to PureCube Thiol-Activated MagBeads via free thiol groups (e.g. cysteine residues) in the protein. Proteins are coupled covalently and can be used for different applications, such as pull-down of interaction partners. Upon coupling of the protein moiety, a 2-thiopyridone group is released from the matrix. Because of the nature of the disulfide bond, the coupled proteins can also be eluted from the Agarose using reducing agents such as DTT or beta-mercaptoethanol. Different thiol containing proteins can be eluted by using a combination or concentration gradient of weaker (e.g. L-cysteine, reduced glutathione) and stronger (DTT or mercaptoethanol) reducing agents.

C. Elution of proteins after coupling to PureCube Thiol-Activated MagBeads.

D. Storage of PureCube Thiol-Activated MagBeads.

E. Regeneration of PureCube Thiol-Activated MagBeads with dipyridyl disulphide for reuse.

Amounts given in this protocol are for 1 mL Thiol-Activated MagBeads in a 25% suspension, corresponding to 250 µL pure 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 (e.g. Cube Biotech #16941)
- ☐ Microcentrifuge tubes (2 mL)
- ☐ End-over-end mixer or thermomixer
- ☐ Spectrophotometer

Materials

- ☐ PureCube Thiol-Activated MagBeads (e.g. 50 mg, Cube Biotech #50601)
- ☐ Sodium phosphate
- ☐ Sodium chloride
- ☐ Sodium hydroxide
- ☐ beta-mercaptoethanol or DTT for elution
- ☐ Optional: L-cysteine, glutathione for modified elution
- ☐ Optional: EDTA and/or protease inhibitor

For activation:

- ☐ Sodium borate
- ☐ 2,2`-dipyridyl disulphide

(aliquot provided, or e.g. Merck Milipore cat no. 841109)

For storage:

- ☐ Sodium acetate
- ☐ Ethanol

Solutions and buffers

PBS buffer pH 7.4, 100 mL

Component	Final concentration	Molecular weight (g/mol)	Stock concentration	Amount needed for stock	Stock needed for buffer
NaH_2PO_4	20 mM	119.98	0.5 M	29.99 g/ 500 mL	4 mL
NaCl*	150 mM	58.44	5 M	146.1 g / 500 mL	3 mL
Instructions: Mix components in 80 mL water. Adjust the pH to 7.4 using NaOH and then add water to a total volume of 100 mL. **Important: Degas the buffer prior to use.** Alternatively, 0.1 M Tris-HCL, or acetate buffers at pH 7.0-8.0 with 0.1-0.5 M NaCl can be used. 1 mM EDTA may be added to remove traces of metal ions which may catalyze thiol oxidation.					

Elution buffer, 10 mL

Component	Final concentration	Molecular weight (g/mol)	Stock concentration	Amount needed for buffer
PBS buffer	1x	n.a.	n.a.	10 mL
DTT	10-25 mM	154.25		15.4-38.6 mg
Alternative to DTT: beta-mercaptoethanol	20-50 mM	78.13	(100%=14.3 M)	14-35 µL
Alternative to DTT: reduced glutathione	50 mM	307.32		154 mg
Alternative to DTT: L-cysteine	5-25 mM	121,16		6.1-30.4 mg
Instructions: Dissolve reducing agent in PBS buffer. Use only one reducing agent at a time.				

Activation buffer, 50 mL

Component	Final concentration	Molecular weight (g/mol)	Required amount	Volume
Sodium borate, pH 8.0	50 mM	381.37	953 mg	50 mL
2,2`-dipyridyl disulphide	saturated	220.3		40 mg
Instructions: Dissolve sodium borate in 40 mL ddH_2O, set pH to 8.0 and fill up to 50 mL. Stir overnight to dissolve. The next morning, add dipyridyl disulphide and incubate on an end over end shaker for 2-3 h to dissolve. Pass through a 0.45 µm filter to remove insoluble particles. **Note:** Depending on the pack size, aliquot(s) of 2,2`-dipyridyl disulphide are provided with the Thiol-Activated MagBeads. **Important: Always prepare fresh. Degas the buffer prior to use.**				

MagBead Storage Buffer, 100 mL

Component	Final concentration	Molecular weight (g/mol)	Stock concentration	Amount needed for stock	Stock needed for buffer
Sodium acetate, pH 6.5	20 mM	82.03	0.5 M	20.5 g/ 500 mL	4 mL
Ethanol	20 % v/v		100 %		20 mL
Instructions: Dissolve sodium acetate in 60 mL water. Adjust the pH to 6.5 using NaOH. Add 20 mL ethanol and add water to a total volume of 100 mL.					

Procedure

A. Activation

1. Pipet 1 mL PureCube Thiol-Activated MagBeads into a 2 mL microtube.
2. Place the tube on a magnetic stand and allow the beads to separate. Remove the supernatant. Resuspend the magnetic beads with 1 ml PBS Buffer.
3. Repeat step 2 to wash the magbeads.
4. Resuspend with 1.5 mL Activation Buffer. Mix by vortexing. Incubate at room temperature for 30 min on an end-over-end shaker or thermoshaker.
5. Remove the supernatant and wash twice with 1 mL PBS Buffer each.
6. Proceed directly to a coupling reaction (Procedure B).

Note: For optimal results, MagBeads should be resuspended directly before use in the coupling reaction.

B. Protein coupling

1. Remove the PBS buffer solution and add 1 mL PBS containing your protein of interest to the MagBeads. Mix by vortexing.
2. Depending on the temperature stability of the protein, incubate at room temperature or 4°C for 30 min on an end-over-end shaker or thermoshaker.
3. Place the tube on a magnetic stand and allow the beads to separate. Remove the supernatant.
4. Wash twice with 1 ml PBS buffer.

Note: Ensure that MagBeads are freshly activated before use.

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.

Note: Incubation time may be extended to 60 min if required.

Tip: To monitor protein coupling efficiency, analyze the supernatant in a spectrophotometer at 280 nm. Note that upon protein coupling, 2-thiopyridone is eluted which absorbs at 280 and 343 nm with similar extinction coefficents of 8.08×10^3 M^{-1} cm^{-1}. Alternatively, 2-thiopyridone can be washed off the MagBeads with a low concentration of reducing agent (e.g. 5 mM DTT or mercaptoethanol) before eluting the protein of interest. However, please check this wash fraction by SDS-PAGE to ensure your protein of interest is not eluted under these conditions.

Procedure

C. Protein elution

1. After protein coupling, resuspend the magnetic beads with 750 µL Elution Buffer. Mix by vortexing.
2. Place the tube on a magnetic stand and allow the beads to separate. Remove the supernatant.
3. Repeat steps 1+2, and analyze the elution fractions by SDS-PAGE.
4. Optional: Repeat steps 1+2 with different types and/or concentrations of reducing agents in the elution buffer.

Note: The supernatant contains the eluted protein. Depending on the type of protein, and available number of thiol groups, the type and concentration of reducing agents may vary.

D. Storage

1. After elution, wash the beads twice with PBS buffer.
2. Add 1 mL MagBead Storage buffer. The Magbeads can be stored at 4°C for several months.

E. Regeneration

1. Wash the magnetic beads twice with 1 ml PBS Buffer each.
2. Resuspend the magnetic beads with 1 mL of Elution Buffer. Incubate for 1 h at room temperature on an end-over-end shaker.
3. Place the tube on a magnetic stand and allow the beads to separate. Remove the supernatant.
4. Wash the magnetic beads twice with 1 mL PBS Buffer each.
5. Resuspend the magnetic beads in 1.5 mL Activation Buffer. Mix by vortexing. Incubate at room temperature for 30 min on an end-over-end shaker or thermoshaker.
6. Place the tube on a magnetic stand and allow the beads to separate. Remove the supernatant.
7. Wash the MagBeads twice with 1 mL PBS Buffer each.
8. Proceed directly to a coupling reaction (Procedure B).

Note: Regenerate Thiol-Activated MagBeads immediately before the next use in a coupling reaction.