# Coupling of proteins to PureCube Maleimide-Activated Agarose

## Overview

This protocol delineates a coupling procedure for proteins to PureCube Maleimide-Activated Agarose. 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 of a 50% Maleimide-Activated Agarose suspension, corresponding to 500 µL agarose bed volume. The process can be linearly scaled up and down from 100 µL to several 100 mL bed volume.
Separation of Agarose and supernatants in low mL scale can be done by centrifugation, or, more conveniently, using PureCube 1-step batch Columns. See the separate protocol available for a combination of these products. For larger scales, use of special equipment like glass vaccum tank filters, is recommended.

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

- ☐ Centrifuge for 15 mL tubes
- ☐ Centrifuge tubes (15 mL)
- ☐ End-over-end mixer or thermomixer
- ☐ Spectrophotometer

## Materials

- ☐ PureCube Maleimide-Activated Agarose (10 mL, Cube Biotech #51103)
- ☐ 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 |
|---|---|---|---|---|
| $NaH_2PO_4$ 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.<br>**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.<br>**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 Agarose suspension (corresponding to 1 mL bed volume) into a 2 ml microcentrifuge tube.
2. Spin the tube at 500 x g to pellet the agarose. Remove the supernatant.
3. Wash the Activated Agarose three times with 1 ml coupling buffer I or II.
4. Resuspend 1-3 mg protein in 500 µl coupling buffer I or II.
5. Add the protein to the activated agarose 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. Spin the tube at 500 x g to pellet the agarose. Remove the supernatant and analyze the supernatant in a spectrophotometer. Record absorption at 280 nm to monitor coupling efficiency.
8. Wash the agarose three times with 1 ml coupling buffer and once with 1 ml double distilled water.
9. Resuspend the coupled Agarose in 1 mL Storage buffer I or II, yielding a 50% 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.