Coupling of proteins to PureCube Epoxy-Activated Agarose

Overview

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

Depending on the proteins to be coupled, two coupling buffers are described at pH 7.2 and 8.3, 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.

Amounts given in this protocol are for 1 mL of a 50% Epoxy-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 Epoxy-Activated Agarose (10 mL, Cube Biotech #50703)
- ☐ Sodium dihydrogen phosphate
- ☐ Sodium chloride
- ☐ Sodium acetate trihydrate
- ☐ Sodium hydroxide (NaOH)
- ☐ Acetic acid
- ☐ Ethanol
- ☐ Tris
- ☐ Optional: Sodium carbonate monohydrate
- ☐ Optional: Ethanolamine

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	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.				

Coupling Buffer II pH 8.3, 250 mL

Component	Final concentration	Molecular weight (g/mol)	Stock concentration	Amount needed for buffer
$NaCO_3$	100 mM	124.00	n.a.	3.1 g
NaCl	500 mM	58.44	n.a.	7.315 g
Instructions: Dissolve components in 200 mL water, adjust the pH to 8.3 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
Tris	1 M	121.1	n.a.	30.29 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.				

Alternative 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.				

Agarose 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 Epoxy-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 four times with 1 ml double distilled water, then wash once with 1 ml coupling buffer I or II.
4. Resuspend the Agarose in 500 µl coupling buffer and incubate for 10 minutes at 20 or 37°C, depending on the stability of your protein.
5. Add the protein to be coupled to the mix. The exact protein amount needs to be optimized, and 5 to 10 mg protein is a good starting point.
6. Incubate at 20-37°C for 24 hours 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 five times with 1 ml double distilled water and once with 1 ml Storage buffer, yielding a 50% suspension. Store at 4°C.
9. Optional: Add 500 µl Quenching Buffer and incubate again for 8 h at 20-37°C. Wash five times with 1 mL double distilled water each and once with 1 ml Storage buffer.
10. Resuspend the coupled Agarose in 1 mL Storage buffer, 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: 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: Choose the highest temperature the protein is stable at. Coupling efficiency increases with temperature, incubation time, and protein concentration.

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 carboxy groups are left on the agarose matrix that might interfere with subsequent assays.