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Coupling Procedure for PureCube Amine Activated Agarose

Product	Catalog No.	Package size
PureCube Amine Activated Agarose (10 mL)	**51003**	**20 mL 50% suspension**
PureCube Amine Activated Agarose (50 mL)	**51005**	**100 mL 50% suspension**
PureCube Amine Activated Agarose (250 mL)	**51010**	**500 mL 50% suspension**
PureCube Amine Activated Agarose (500 mL)	**51012**	**1000 mL 50% suspension**

Chemicals and buffers

Important: Never use buffers with free amines (e.g. tris) or carboxylate groups in EDC/NHS coupling reactions.
Depending on the nature of the protein, binding and wash buffers I or II might give best results.

Coupling buffer I (PBS): 150 mM Na phosphate, 100 mM NaCl, pH 7.2
Coupling buffer II: 0.1 M MES, 150 mM NaCl, pH 4.7

Wash buffer I (PBS): 150 mM Na phosphate, 100 mM NaCl, pH 7.2
Wash buffer II: 250 mM NaCl

Storage buffer I: 20 mM sodium acetate pH 6,5, 20% Ethanol
Storage buffer II: 100 mM sodium hydrogen carbonate, 0.02% sodium azide, pH 7.5

EDC: ((1-Ethyl-3-(3-dimethylaminopropyl)carbodiimide) is hygroscopic and immediately starts hydrolyzing when getting in contact with humidity. It is highly recommended to open EDC bottles under protective gas and to let the EDC equilibrate to room temperature before opening. Use EDC directly after withdrawal from storage vessel!
Important: If you are not sure if the EDC has been in contact with humidity, discard the material and use fresh EDC.

Coupling Procedure

This protocol was established for coupling of proteins to 1 ml agarose beads (corresponding to 2 ml of a 50% suspension) in a 15 ml tube. The reaction can be scaled up or down linearly if required.
Separation of Agarose and supernatants in low mL scale can be done by centrifugation, or, more conveniently, using PureCube 1-step batch Columns. For larger scales, use of special equipment like glass vaccum tank filters, is recommended.

Dispense 2 ml of the 50% agarose suspension into a 15 ml plastic tube. Pellet the agarose by centrifugation at 500 x g, and remove the supernatant. Wash the agarose three times with 5 ml binding buffer I each.
Resuspend 2-7 mg protein in 1 ml binding buffer I, add them to the agarose suspension and mix by vortexing. Incubate the reaction mixture for 5-10 minutes at 4°C on an end-over-end shaker.

Equilibrate the EDC to room temperature (see above) and add 15 mg EDC directly to the suspension and mix immediately by vortexing. Incubate for 1h at room temperature – or for 2 h at 4°C if the protein is temperature-sensitive- on an end-over-end shaker.

In some cases, the binding capacity can be raised by a second addition of 10 mg EDC and additional 1-2h incubation.

Centrifuge the solution at 500 x g and discard the supernatant. Wash the agarose six times with 5 ml Wash Buffer I each. Add 1 ml storage buffer to obtain a 50% suspension for storage at 4°C and further use.

Please contact us (contact@cube-biotech.com) if you have any questions or need assistance optimizing a protocol for your application.