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Coupling Procedure for PureCube Amine Activated MagBeads

Product	Catalog No.	Package size
PureCube Amine Activated MagBeads (1 mL)	**50901**	**1 x 1 mL 25% suspension**
PureCube Amine Activated MagBeads (5 mL)	**50905**	**1 x 5 mL 25% suspension**
PureCube Amine Activated MagBeads (25 mL)	**50925**	**1 x 25 mL 25% suspension**
PureCube Amine Activated MagBeads (4x25 mL)	**50990**	**4 x 25 mL 25% 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 200 µl pure magnetic beads (corresponding to 800 µl of a 25% suspension) in a 2 ml microtube. The reaction can be scaled up or down linearly if required.

Dispense 800 µl of the 25% magbead suspension into a 2 ml microtube and place the tube in a magbead separator. Remove the supernatant and wash the magbeads three times with 1 ml PBS each.
Resuspend 400 µg to 2 mg protein in 200 µl PBS, add them to the suspension and mix by vortexing. Incubate the reaction mixture for 5-10 minutes at 4°C on an end-over-end shaker.

Add **a freshly prepared** solution of 4 mg EDC (1-Ethyl-3-(3-dimethylaminopropyl)carbodiimide) in 200 µl PBS and mix well by vortexing. Depending on the stability of your protein, incubate for 2h at 4°C or 1h at room temperature on an end-over-end shaker.
In some cases, the binding capacity can be raised by a second addition of 4 mg EDC and additional 1-2h incubation.

Place the microtube into a magnetic separator and discard the supernatant. Wash the mag beads five times with 1 ml PBS and once with 1 ml double distilled water. Resuspend the magbeads in 800 µl storage buffer. Keep at 4°C until further use.

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