

Toll Free: 866-252-7771 Phone: 732-469-7771 Fax: 732-469-7782 Web: www.purebiotechllc.com

Products for Biotechnology

With Magnetic Porous Glass (MPG®)

Protocol No.:3.108Product:MPG* Long Chain Alkylamine (30 mg/ml, 1.2 - 1.8 × 10^a particles/ml)Procedure:Covalent Attachment of Proteins.Storage:Ambient Temperature

PRODUCT NUMBER MLCA0502 MLCA0510

DESCRIPTION MPG® LCA, 5 µm, 50 nm (500 Å) pore diameter VOLUME 2 ml (60 mg) 10 ml (300 mg)



*TO COUPLE OLIGONUCLEOTIDES SEE PROTOCOL 3.2

General Procedure

Materials: (Based on 10 mg MPG® Long Chain Alkylamine, suspended in 10 mM phosphate, pH 7.5, 0.15 M NaCl)

Protein of Interest 25% Glutaraldehyde (CHO(CH₂)₃CHO) Sodium Cyanoborohydride (NaBH₃CN) Sodium Azide (NaN₃) Deionized Water (dH₂O) Glycine (H₂NCH₂COOH) 2N Hydrochloric Acid (HCl) Sodium Chloride (NaCl) Bovine Serum Albumin (BSA) Sodium Phosphate, Monobasic (NaH₂PO₄) Sodium Phosphate, Dibasic, Heptahydrate (Na₂HPO₄) Magnetic Particle Separator, Prod.No.MPS0301 or MPS0001 Low Speed Rotator 1.5 ml Microcentrifuge Tubes Pipette and Pipette Tips Vortex Mixer

<u>Solution</u> Coupling Buffer (10 mM Phosphate, pH 7.5)	$\label{eq:preparation} \frac{\text{Preparation}}{\text{Dissolve 19.2 mg NaH_2PO_4 and 225.2 mg Na_2HPO_4} \cdot 7H_2O \text{ in 80 ml dH_2O}.$ Adjust to pH 7.5 with 2N HCl, if necessary, and bring volume to 100 ml with dH_2O.
Activation Solution (5% Glutaraldehyde)	Add 0.2 ml 25% Glutaraldehyde to 0.8 ml Coupling Buffer.
1% Sodium Cyanoborohydride Solution (Fresh)	Dissolve 10 mg NaBH ₃ CN in 1 ml Coupling Buffer.
0.75% Glycine Solution	Dissolve 7.5 mg Glycine in 1 ml Coupling Buffer.
Wash Buffer (10 mM Phosphate, pH 7.5, 1.0 M NaCl)	Dissolve 584.7 mg NaCl in 8 ml of Coupling Buffer. Bring to 10 ml with Coupling Buffer.
Storage Buffer (10 mM Phosphate, pH 7.5, 150 mM NaCI, 0.1% BSA, 0.02% NaN₃)	Dissolve 87.7 mg NaCl, 10 mg BSA and 2 mg NaN ₂ in 8 ml of Coupling Buffer. Bring to 10 ml with Coupling Buffer.

Activation of MPG[®] Long Chain Alkylamine

- 1. Adjust the concentration of MPG[®] Long Chain Alkylamine to 10 mg/ml. Transfer 1 ml to a 1.5 ml microcentrifuge tube. Magnetically separate the MPG[®] Long Chain Alkylamine from the solution by placing the tube in a Magnetic Particle Separator for at least 30 seconds. Remove the supernatant by aspiration while the tube remains in the particle separator.
- 2. Add 1 ml of Coupling Buffer and mix well. Magnetically separate and aspirate the supernatant.
- 3. Add 1 ml of Activation Solution to the MPG[®] Long Chain Alkylamine particles, mix well and place in a low speed rotator for 1½ hours at room temperature. Magnetically separate and aspirate the supernatant.
- 4. Add 1 ml of Coupling Buffer to the activated MPG* Long Chain Alkylamine particles and mix well. Magnetically separate and remove the supernatant. Repeat this step four more times.

Coupling of Protein to Activated MPG® Long Chain Alkylamine

 Dissolve 2.5 mg Protein in 1 ml of Coupling Buffer.* Add this mixture and 50 µl of 1% Sodium Cyanoborohydride Solution to the activated MPG* Long Chain Alkylamine particles. Mix well and rotate 3 hours at room temperature. Magnetically separate and aspirate the supernatant.

*THE CONCENTRATION OF THE SPECIFIC PROTEIN SHOULD BE TITRATED TO ACHIEVE OPTIMAL COUPLING TO THE PARTICLE SURFACE.

- 2. Add 1 ml of 0.75% Glycine Solution and 50 µl of 1% Sodium Cyanoborohydride Solution, mix well and rotate 1 hour at room temperature. Magnetically separate and aspirate the supernatant.
- 3. Add 1 ml of Washing Buffer and mix well. Magnetically separate and remove the supernatant. Repeat this step four more times. The protein-bound MPG[®] Long Chain Alkylamine is ready to use.

4. For storage, add 1 ml of Storage Buffer to the protein-bound MPG[®] Long Chain Alkylamine and mix well. Magnetically separate and aspirate the supernatant. Resuspend the protein-bound MPG[®] Long Chain Alkylamine particles in 1 ml Storage Buffer and store at 4°C.

FOR TECHNICAL SERVICE ON THIS OR ANY OTHER PureBiotech PRODUCT CALL 866-252-7771 or e-mail us at info@purebiotechllc.com.

For in vitro research use only.

MPG[®] is a registered trademark of Millipore Corporation. Magnetic Porous Glass and certain applications in which it is used are covered by U.S. Patent 5,601,979; 5,610,274 and 5,734,020 owned by Millipore Corporation and licensed by PureBiotech, LLC.