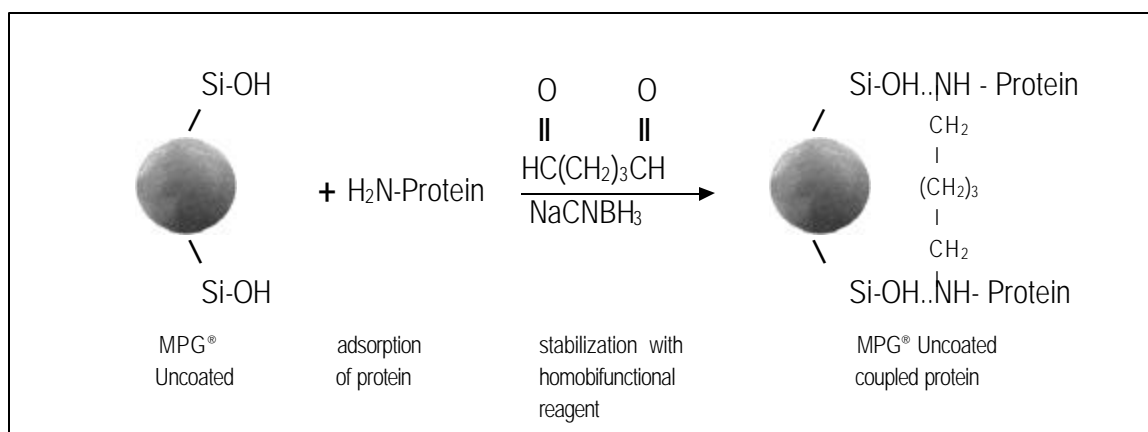


Products for Biotechnology

With Magnetic Porous Glass (MPG®)

Protocol No.: 1.106
Product: MPG® Uncoated (30 mg/ml, 1.2 - 1.8 × 10⁸ particles/ml)
Procedure: Attachment of Proteins
Storage: Ambient Temperature

PRODUCT NUMBER	DESCRIPTION	VOLUME
MCPG0502	MPG® Uncoated, 5 µm, 50 nm (500 Å) pore diameter	2 ml (60 mg)
MCPG0510		10 ml (300 mg)



General Procedure

Materials: (Based on 10 mg MPG® Uncoated, suspended in water)

Protein of Interest	Sodium Chloride (NaCl)
Glycine (H ₂ NCH ₂ COOH)	Bovine Serum Albumin (BSA)
25% Glutaraldehyde (CHO(CH ₂) ₃ CHO)	Deionized Water (dH ₂ O)
2N Hydrochloric Acid (HCl)	1.5 ml Microcentrifuge Tubes
Sodium Cyanoborohydride (NaBH ₃ CN)	Magnetic Particle Separator, Prod. No. MPS0301 or MPS0001
Sodium Phosphate, Monobasic (NaH ₂ PO ₄)	Low Speed Rotator
Sodium Phosphate, Dibasic, Heptahydrate (Na ₂ HPO ₄)	Pipettes and Pipette Tips
Sodium Azide (NaN ₃)	Vortex Mixer

Solution

Coupling Buffer
(100 mM Phosphate, pH 7.5)

5% Glutaraldehyde Solution

1% Sodium Cyanoborohydride
Solution (Fresh)

Preparation

Dissolve 192 mg NaH₂PO₄ and 2.252 g Na₂HPO₄·7H₂O in 80 ml dH₂O.
Adjust to pH 7.5 with 2N HCl, if necessary. Bring volume to 100 ml with dH₂O.

Add 1 ml of 25% Glutaraldehyde to 4 ml Coupling Buffer.

Dissolve 10 mg NaBH₃CN in 1 ml Coupling Buffer.

Solution *(continued)*

0.75% Glycine Solution

Washing Buffer
(10 mM Phosphate, pH 7.5,
1 M NaCl, 0.1% BSA)

Storage Buffer
(10 mM Phosphate, pH 7.5,
150 mM NaCl, 0.1% BSA,
0.02% NaN₃)

Preparation *(continued)*

Dissolve 7.5 mg Glycine in 1 ml Coupling Buffer.

Dilute 10 ml of Coupling Buffer in 50 ml of dH₂O. Add 5.84 g NaCl and 100 mg BSA. Adjust volume to 100 ml with dH₂O.

Dilute 10 ml Coupling Buffer with 80 ml of dH₂O. Add 877 mg NaCl, 100 mg BSA and 20 mg NaN₃. Bring volume to 100 ml with dH₂O.

Protein Adsorption

1. Adjust the concentration of MPG® Uncoated to 10 mg/ml and add 1 ml to a 1.5 ml microcentrifuge tube. Magnetically separate the MPG® Uncoated from the solution by placing the tube in a Magnetic Particle Separator for at least 30 seconds. Carefully remove the supernatant by aspiration with a pipette while the tube remains in the particle separator.
2. Add 1 ml of Coupling Buffer to MPG® Uncoated and mix well. Magnetically separate the MPG® Uncoated from the solution and remove the supernatant. Repeat this step two more times.
3. Dissolve 2.5 mg Protein of Interest in 1 ml of Coupling Buffer.* Add this protein solution to the MPG® Uncoated particles and mix well. Place the tube in a Low Speed Rotator and mix for 16-24 hours at room temperature. Magnetically separate and remove the supernatant by aspiration.

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

4. Add 1 ml of Coupling Buffer to the protein-bound MPG® Uncoated and mix well. Magnetically separate and remove the supernatant.

Stabilization of Proteins

1. Mix 100 µl of 5% Glutaraldehyde Solution with 900 µl Coupling Buffer and add to the protein-bound MPG® Uncoated particles. Add 50 µl of 1% Sodium Cyanoborohydride Solution to wet protein-bound MPG® Uncoated particles, mix well, place the tube in a Low Speed Rotator and mix for 3 hours at room temperature. Magnetically separate and aspirate the supernatant.
2. Add 500 µl of Coupling Buffer, 500 µl of 0.75% Glycine Solution and 50 µl of 1% Sodium Cyanoborohydride Solution to protein-bound MPG® Uncoated particles. Mix well and rotate one hour at room temperature. Magnetically separate and aspirate the supernatant.
3. Add 1 ml of Washing Buffer to protein-bound MPG® Uncoated and mix well. Magnetically separate and aspirate the supernatant. Repeat this step four more times. Protein-bound MPG® Uncoated is now ready to use.
4. For storage, add 1 ml of Storage Buffer to protein-bound MPG® Uncoated and mix well. Magnetically separate and aspirate the supernatant. Resuspend the protein-bound MPG® Uncoated particles in 1 ml of 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.

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