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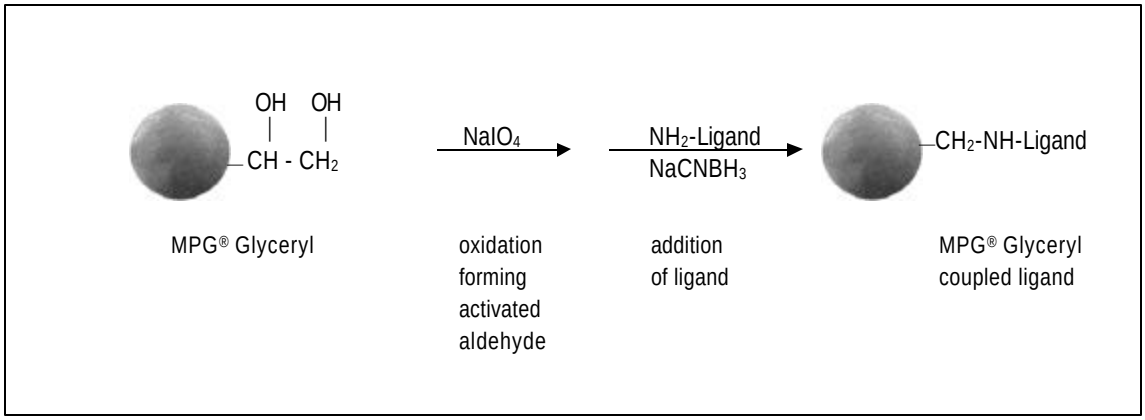
Products for Biotechnology

With Magnetic Porous Glass (MPG®)

Protocol No.: **2.105**
Product: **MPG® Glyceryl** (30 mg/ml, 1.2 - 1.8 × 10^8 particles/ml)
Procedure: **Covalent Attachment of Biomolecules with Alkylamine Groups.**
Storage: **Ambient Temperature**

PRODUCT NUMBER	DESCRIPTION	VOLUME
MGLY0502	MPG® Glyceryl, 5 µm, 50 nm (500 Å) pore diameter	2 ml (60 mg)
MGLY0510		10 ml (300 mg)



General Procedure

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

- Protein or Biomolecule of Interest
- Sodium Phosphate, Monobasic (NaH_2PO_4)
- Sodium Phosphate, Dibasic, Heptahydrate (Na_2HPO_4)
- Sodium Azide (NaN_3)
- Sodium Chloride (NaCl)
- 2N Hydrochloric Acid (HCl)
- Glycine (H_2NCH_2COOH)
- Deionized Water (dH_2O)
- Sodium Cyanoborohydride ($NaBH_3CN$)
- Bovine Serum Albumin (BSA)
- Sodium Periodate ($NaIO_4$)
- 1.5 ml Microcentrifuge Tubes
- Magnetic Particle Separator, Prod. No. MPS0301 or MPS0001
- Low Speed Rotator
- Vortex Mixer
- Pipettes and Pipette Tips

Solution	Preparation
Coupling Buffer (10 mM Phosphate, pH 7.5)	Dissolve 19.2 mg NaH_2PO_4 and 225.2 mg $Na_2HPO_4 \cdot 7H_2O$ in 80 ml dH_2O. Adjust to pH 7.5 with 2 N HCl, if necessary, and bring volume to 100 ml with dH_2O.
Activation Solution (150 mM Sodium Periodate)	Dissolve 32 mg $NaIO_4$ in 1 ml dH_2O.

Solution *(continued)*	**Preparation***(continued)*
0.75% Glycine Solution	Dissolve 7.5 mg Glycine in 1 ml of Coupling Buffer.
1% Sodium Cyanoborohydride Solution (Fresh)	Dissolve 10 mg $NaBH_3CN$ in 1 ml of Coupling Buffer.
Washing Buffer (10 mM Phosphate, pH 7.5, 1.0 M NaCl)	Dissolve 584.7 mg NaCl in 5 ml of Coupling Buffer. Bring to 10 ml with Coupling Buffer.
Storage Buffer (10 mM Phosphate, pH 7.5, 150 mM NaCl, 0.1% BSA, 0.02% NaN_3)	Dissolve 87.7 mg NaCl, 10 mg BSA and 2 mg NaN_3 in 8 ml Coupling Buffer. Bring to 10 ml with Coupling Buffer.

Activation of MPG® Glyceryl

1. Adjust the concentration of MPG® Glyceryl to 10 mg/ml. Transfer 1 ml to a 1.5 ml microcentrifuge tube. Magnetically separate the MPG® Glyceryl 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. Remove the tube from the particle separator. Add 1 ml of Coupling Buffer and mix well. Magnetically separate the MPG® Glyceryl from the solution and aspirate the supernatant.

3. Add 1 ml of Activation Solution to the MPG® Glyceryl and mix well. Place the tube in a low speed rotator and rotate 1½ hours at room temperature. Magnetically separate and aspirate the supernatant.

4. Add 1 ml of Coupling Buffer to the activated MPG® Glyceryl and mix well. Magnetically separate and remove the supernatant. Repeat this step four more times.

Coupling of Protein to Activated MPG® Glyceryl

1. Dissolve 2.5 mg of Protein (for Antibody use 1 mg) or Biomolecule of Interest in 1 ml of Coupling Buffer.* Add this mixture and 50 µl of the 1% Sodium Cyanoborohydride Solution to the activated MPG® Glyceryl. Mix well and rotate 3 hours at room temperature. Magnetically separate and aspirate the supernatant.

   ***THE CONCENTRATION OF THE SPECIFIC BIOMOLECULE 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 or biomolecule-bound MPG® Glyceryl is ready to use.

4. For storage, add 1 ml Storage Buffer to the protein or biomolecule-bound MPG® Glyceryl and mix well. Magnetically separate and aspirate the supernatant. Resuspend the protein or biomolecule-bound MPG® Glyceryl particles in 1 ml Storage Buffer and store at 4°C.

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