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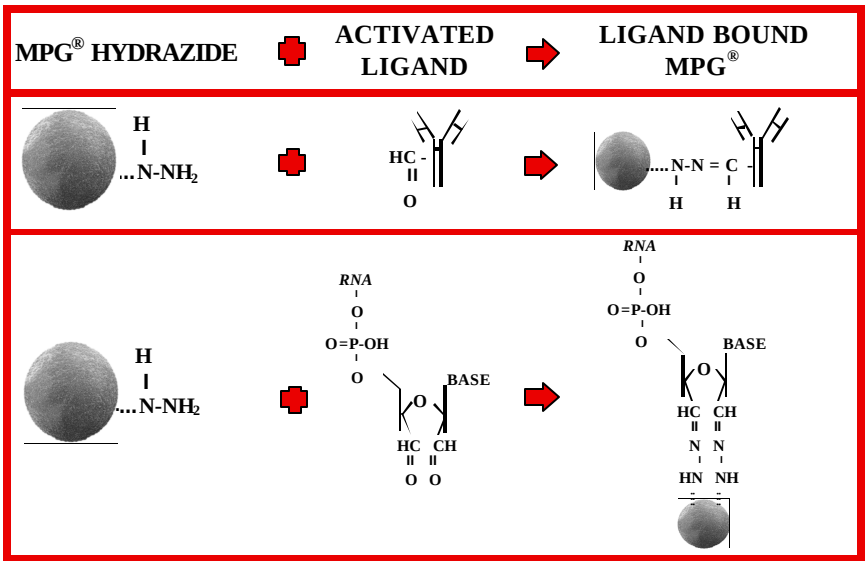
Products for Biotechnology

With Magnetic Porous Glass (MPG®)

Protocol No.: 9.105
Product: MPG® Hydrazide (30 mg/ml, 1.2 - 1.8 x 10^8 particles/ml)
Procedure: Covalent Attachment of Glycoproteins
Storage: Ambient Temperature

PRODUCT NUMBER	DESCRIPTION	VOLUME
MHDZ0502	MPG® Hydrazide, 5 µm, 50 nm (500 Å) pore diameter	2 ml (60 mg)
MHDZ0510		10 ml (300 mg)



General Procedure

Materials: (Based on 10 mg MPG® Hydrazide, suspended in PBS, pH 7.5, 0.02% NaN_3)

- Glycoprotein of Interest (<10 mg/ml)
- Sodium Acetate (CH_3COONa)
- Sodium Chloride (NaCl)
- Sodium Periodate ($NaIO_4$)
- Sodium Phosphate, dibasic (Na_2HPO_4)
- Potassium Chloride (KCl)
- Potassium Phosphate, monobasic (KH_2PO_4)
- Magnesium Chloride ($MgCl_2$)
- Glycerol ($HOCH_2CHOHCH_2OH$)
- Glyceraldehyde D or L ($HOCH_2CHOHCHO$)
- Deionized Water (dH_2O)
- Hydrochloric Acid (HCl)
- Acetic Acid (CH_3COOH)
- Tween 20 or Triton X-100
- Dialysis Tubing (MW cutoff 10,000)
- Stirring Plate with Spin Bars
- Magnetic Particle Separator, Prod No. MPS0301 or MPS0001
- Low Speed Rotator
- Microcentrifuge Tubes
- Vortex Mixer

Solution	Preparation
Oxidation Buffer (100 mM Sodium Acetate, 150 mM NaCl, pH 5.5)	Dissolve 8.2 g Sodium Acetate and 8.76 g NaCl in 800 ml dH_2O. Adjust pH to 5.5 with Acetic Acid and bring the volume to 1000 ml with dH_2O.
Activation Solution (467 mM Sodium Periodate)	Dissolve 1 g Sodium Periodate in 10 ml dH_20.
Quenching Solution (50% Glycerol in Oxidation Buffer)	Mix 5 ml of 100% Glycerol with 5 ml Oxidation Buffer.
Coupling Buffer (100 mM Sodium Acetate, 150 mM NaCl, pH 4.5)	Dissolve 8.2 g Sodium Acetate and 8.76 g NaCl in 800 ml dH_2O. Adjust pH to 4.5 with Acetic Acid and bring the volume to 1000 ml with dH_2O.
Wash/Storage Buffer (PBS: 138 mM NaCl, 2.7 mM KCl, 8.1 mM Na_2HPO_4, 1.2 mM KH_2PO_4, 0.5 mM $MgCl_2$ + 0.1% Tween 20)	Dissolve 8 g NaCl, 0.2 g KCl, 1.15 g Na_2HPO_4, 0.20 g KH_2PO_4 and 0.047 g $MgCl_2$ in 800 ml dH_2O. Adjust pH to 7.4 with HCl and bring volume to 1000 ml with dH_2O. Add 1 ml Tween 20. NOTE: Triton X-100 can be substituted for Tween 20.
Capping Solution (67 mM Glyceraldehyde in Coupling Buffer)	Dissolve 6 mg Glyceraldehyde per 1 ml Coupling Buffer. Make fresh for each reaction.

ALL PROCEDURES ARE TO BE PERFORMED AT 4°C

Oxidation and Activation of Glycoprotein

NOTE 1: THIS PROCEDURE IS OPTIMIZED FOR GLYCOPROTEINS THAT CAN TOLERATE 10 mM SODIUM PERIODATE AND ARE STABLE AT pH 5.5. FOR GLYCOPROTEINS SENSITIVE TO PERIODATE USE 1.0 mM SODIUM PERIODATE IN THE ACTIVATION SOLUTION. DO NOT USE THIS PROTOCOL FOR GLYCOPROTEINS THAT PRECIPITATE AT pH 5.5 SUCH AS IgM 1D3.

1. Dialyze the glycoprotein against Oxidation Buffer, 2 x 1,000 volumes overnight. (Make sure the concentration of the glycoprotein is < 10 mg/ml)

2. Chill the dialyzed glycoprotein on ice and add Activation Solution to a final concentration of 10 mM (22 µl/ml). Incubate IN THE DARK for 1 hour at 4°C.

3. Quench oxidation with 2.2 µl/ml Quenching Solution (final concentration 100 mM). Incubate IN THE DARK ON ICE for 30 minutes.

4. Dialyze the activated glycoprotein against 2 x 1,000 volumes of Coupling Buffer overnight at 4°C.

Coupling of Activated Glycoprotein to MPG® Hydrazide

NOTE 2: COUPLING EFFICIENCIES TO MPG® HYDRAZIDE WILL BE DEPENDENT ON THE TERTIARY STRUCTURE AND SIZE OF THE PARTICULAR GLYCOPROTEIN TO BE COUPLED. THEREFORE, OPTIMIZATION OF THE GLYCOPROTEIN CONCENTRATION, BY TITRATION, IS RECOMMENDED. FOR IgG IMMUNOASSAYS, THE OPTIMAL CONCENTRATION HAS BEEN FOUND TO BE 25-50 µg/ml. FOR LIGAND PURIFICATION TRY 75-150 µg/ml.

1. Transfer 10 mg MPG® Hydrazide (or the appropriate amount) to a microcentrifuge tube. Magnetically separate the particles by placing the tube in a Magnetic Particle Separator for 1 - 2 minutes. Gently remove the supernatant.

2. Add 1 ml of Coupling Buffer to the particles and mix well (a vortex may be used). Magnetically separate and remove the supernatant. Repeat 2 more times.

3. Add 500 µl of the oxidized glycoprotein (at the appropriate concentration: i.e. 25 - 50 µg IgG per ml (see NOTE 2 above)) to the 10 mg of MPG® Hydrazide. Incubate overnight at room temperature with a mixing rotator.

4. Magnetically separate and wash 3 times with 500 µl Coupling Buffer. Magnetically separate and remove the supernatant.

5. Add 100 µl Capping Solution to the washed particles and incubate with a low speed rotator for 30 - 60 minutes. Magnetically separate and remove the supernatant

6. Add 500 µl Wash/Storage Buffer and mix well. Magnetically separate and remove the supernatant. Repeat this step 9 more times. Glycoprotein-bound MPG® Hydrazide is now ready to use.

7. For storage, add 1 ml Wash/Storage Buffer to the glycoprotein-bound MPG® Hydrazide and store at 4°C.

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