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Products for Biotechnology
With Magnetic Porous Glass (MPG®)

Protocol No.: 67.103
Product: MPG® Protein G (10 mg/ml, 4-6 x 10^7 particles/ml)
Procedure: Isolation and Purification of Immunoglobulins
Storage: 2-8ºC, Do Not Freeze.
Stability: Performance of this product is guaranteed for 6 months from date seal is broken.

PRODUCT NUMBER	DESCRIPTION	VOLUME
MPGPG0501	MPG® Protein G, 5 µm, 500 Å pore diameter	1 ml (10 mg)
MPGPG0505		5 ml (50 mg)
MPGPG0510		10 ml (100 mg)

Principle and Applications

MPG® Protein G is a suspension of spheroidal totally porous borosilicate glass particles embedded with superparamagnetic iron oxide to which highly purified recombinant Protein G has been covalently attached.

The recombinant Protein G used in this product is Gammabind G Type II (1), a truncated form of Protein G with a molecular mass of 22,600 daltons that migrates on SDS-PAGE with an apparent mass of ~32,000 daltons because of the extended structure of the protein. The albumin and Fab binding sites (2) present in the amino terminus as well as the hydrophobic membrane anchor region in the carboxyl terminus of the full length protein(3) have been removed. This form of Protein G has two binding sites that are very specific for the F(c) region of immunoglobulin class gamma (IgG). The chemistry used for conjugating protein G to MPG favors attachment to a lysine rich region (through a very stable alkyl-imide linkage) that leaves these binding sites available for interaction with IgG. This allows maximum biological activity of the bound antibody by orienting its constant region towards the particle while giving the variable region full access to antigen. Immunoglobulin binding is very strong (K_a=10^9 to 7 x 10^{10}) yet reversible at low pH (4).

These properties provide a simple yet powerful scheme when combined with our magnetic particle technology. This product works well for both small scale affinity purification as well as immunoassays and bioassays directly in ascites, serum, homogenates, lysates, and culture supernatants. In these applications, the use of magnetic particles eliminates the need for centrifugation, spin baskets, and columns. This format reduces assay/purification time by promoting rapid, gentle and complete washing involving few steps and a single tube. Magnetic particle technology also allows the purified target protein to be recovered in as little as 10 µl per mg particles. The nominal binding capacity of 1 mg of MPG® Protein G particles is greater than 75 µg of rabbit IgG. See product specification sheet for lot specific information.

Guidelines for Optimal Use

Protein G has varying affinity for different immunoglobulins of class gamma (no binding to other classes) as shown in Table 1 below. Most sera can be used without concern. However, polyclonal immunoglobulin from chicken, cat, dog, pig and monoclonal rat IgG_{2b} will either fail to bind or give non-quantitative binding. In these situations we recommend using one of our MPG® Secondary Antibody Products or MPG Protein A as appropriate.

Table 1. Binding specificity of Protein G for immunoglobulins from different species.		
SPECIES	SUBCLASS	AFFINITY OF PROTEIN G
Bovine	IgG1	Strong
	IgG2	Strong
Chicken	IgG	None
Dog	IgG	Weak
Donkey	IgG	Strong
Goat	IgG	Strong (96-121)
	IgG1	Strong
	IgG2	Strong
Guinea Pig	IgG1	Strong
	IgG2	Strong
Horse	IgG	Strong
Human	IgG	Strong (88-100)
	IgG1	Strong
	IgG2	Strong
	IgG3	Strong (83-85)
	IgG4	Strong
Mouse	IgG	Strong (84-90)
	IgG1	Moderate (34-71)
	IgG2a	Strong
	IgG2b	Strong
	IgG3	Strong
Pig	IgG	Weak
Rabbit	IgG	Strong (95-128)
Rat	IgG	Moderate (76-86)
	IgG1	Moderate
	IgG2a	Moderate (42-46)
	IgG2b	Weak
	IgG2c	Strong
Sheep	IgG	Strong
	IgG1	Strong
	IgG2	Strong

[a]The table is adapted from reference 3. Numbers in parentheses refer to binding capacity ranges (µg immunoglobulin/mg particles) obtained in-house for MPG® Protein G using either purified IgG in PBS or 10% serum in PBS

The binding of immunoglobulin to Protein G is impacted by **buffer conditions**. The optimal binding pH is near 5.0, although the binding is strong in the pH range of 4-8. PBS is most commonly used for binding although other buffers can be used.

Other factors affecting the binding reaction include **temperature**, **concentration** of both particles and immunoglobulin and **incubation time**. All these are application specific. Most binding reactions are done at room temperature although other temperatures can be used. The optimal concentrations of immunoglobulin and particles and incubation time should be optimized empirically by the researcher. For example, using 5 to 130 fold excesses of MPG® Protein G particles at room temperature in 1-2 ml PBS, goat IgG can be completely cleared (>95%) within 5 to 30 minutes at concentrations ranging from 100 ug/ml to 100 ng/ml, respectively. Using lower concentrations of particles and/or immunoglobulin, particularly if complexed to antigen may require overnight or longer times for complete binding.

Recovery of immunoglobulin (or immunoglobulin-antigen complex) from the particles can be accomplished over the pH range of 2.5-3.0. Binding activity of immunoglobulin can be destroyed by acidity so it is wise to use the highest effective pH and to neutralize by adding 1 M Tris base or 1 M dibasic phosphate to the recovered immunoglobulin.

We recommend glycine-HCl or acetate buffers for elution. We do not recommend using acidic citrate buffers with MPG® Protein G. Immunoglobulin can be recovered in concentrated form with MPG® Protein G. Using 20 µl of 0.1 M glycine-HCl, pH 2.5 per mg particles will give ~70% recovery of captured immunoglobulin (at concentrations greater than 4 µg/µl for fully loaded particles). For complete recovery of immunoglobulin at least 200 µl of 0.1 M glycine-HCl, pH 2.5 per mg particles is recommended. Elution buffers of higher pH and/or lower ionic strength may give somewhat different results due to the buffering capacity of the particles.

With proper use and storage, MPG® Protein G particles can be reused. Using 10% normal Goat serum the particles can be regenerated up to 12 times with essentially no change in binding capacity. Some particle loss is inevitable during continued reuse. This may appear as an apparent loss in binding capacity. The particle concentration can be adjusted using the following factor: the OD600 of a 1mg/ml suspension of MPG® Protein G particles equals 0.955.

Procedure

Materials: *MPG® Protein G is supplied as a 1.0% suspension containing:*
Buffer: 0.01 M Phosphate, 0.15 M Sodium Chloride, pH 7.4
Stabilizer: 1 mg/ml Bovine Serum Albumin (BSA) – Immunoglobulin and protease free
Preservative: 0.02% (w/v) Sodium Azide

2 N Hydrochloric Acid (HCl)
Sodium Phosphate, Dibasic (Na_2HPO_4)
Potassium Phosphate, Monobasic (KH_2PO_4)
Potassium Chloride (KCl)
Glycine ($C_2H_5NO_2$)
Sodium Chloride (NaCl)
Bovine Serum Albumin (BSA)
Deionized Water (dH_2O)
Sodium Azide (NaN_3)
1.5 ml Microcentrifuge Tubes
Magnetic Particle Separator, Prod. No. MPS0301 or MPS0001
Low Speed Rotator
Pipettes and Pipette Tips
Vortex Mixer
Sample containing immunoglobulin

Solution	Preparation
1M Na_2HPO_4	Dissolve 142 g Na_2HPO_4 in 800 ml dH_2O and adjust volume to 1000 ml with dH_2O, pH should be ~9.0.
PBS (Phosphate-buffered saline pH 7.4, {PBS})	Dissolve 8 g NaCl, 0.2 g KCl, 1.17 g Na_2HPO_4 and 0.24 g KH_2PO_4 in 800 ml dH_2O. Adjust the pH to 7.4 with 2 N HCl and adjust volume to 1000 ml with dH_2O.
Elution Buffer (0.1M Glycine-HCl, pH 2.5)	Dissolve 7.5 g Glycine in 500 ml dH_2O. Using 2 N HCl, adjust pH to 2.5. Adjust volume to 1000 ml with H_2O. (Higher pH Elution Buffer can be substituted if desired.)
Storage Buffer (PBS, pH 7.4, 0.1% BSA, 0.02% NaN_3)	Dissolve 100 mg BSA and 20 mg NaN_3 in 100 ml of PBS.

NOTE: These buffer formulations are only intended as a guide. Other buffers can be substituted as your application demands.

Preparation of MPG® Protein G:

- *The following protocol was developed for use with 1-3 mg of particles. For quantities outside this range the protocol can be scaled accordingly.*

- *This preliminary cycling procedure is usually necessary to give maximum immunoglobulin purity. It will remove various additives including any traces of previously bound immunoglobulin, BSA and anti-microbials from the storage buffer, remove any Protein G leached during prolonged storage, and concentrate the particles*.

1. Vortex the MPG® Protein G to fully suspend the particles. Add the desired amount of particles to a 1.5 ml microcentrifuge tube. Magnetically separate the particles from the solution by placing the tube in a Magnetic Particle Separator for at least 30 seconds and carefully remove the supernatant by aspiration, with a pipette, while the tube remains in the particle separator.

2. Resuspend the MPG® Protein G particles in 0.5 ml PBS and mix well. Magnetically separate and remove the supernatant. Repeat this step one more time.

3. Resuspend the MPG® Protein G particles in 0.5 ml Elution Buffer and mix well. Magnetically separate and remove the supernatant.

4. Resuspend the MPG® Protein G particles in 0.5 ml PBS and mix well. Magnetically separate and remove the supernatant.

Binding of Immunoglobulin to MPG® Protein G:

NOTE: *The following protocol has been developed using 10% serum in PBS (~7.5 mg/ml protein). More concentrated samples and samples containing particulates will require additional washing steps.*

1. Resuspend the particles in an immunoglobulin solution prepared in PBS. The immunoglobulin and particle concentrations should be determined empirically, but can be gauged by the information in the *Guidelines for Optimal Use Section on page1*.

2. Incubate at room temperature for 5 minutes or longer with gentle mixing on low speed rotator (see the *Guidelines for Optimal Use Section – page 1*). Magnetically separate and remove the supernatant.

3. Wash by adding 0.5 ml of PBS to the immunoglobulin-bound MPG® Protein G particles and mix well. Magnetically separate and remove the supernatant. Repeat three more times.

Elution of Immunoglobulin from MPG® Protein G:

Recover immunoglobulin by suspending the complex in 200 µl Elution Buffer (as little as 20 µl may be used, see *Guidelines for Optimal Use Section*) for at least 3 minutes, with gentle mixing, at room temperature. Magnetic particles can then be separated and the supernatant, containing immunoglobulin, can be used in your application. Repeat this step if an additional 10% recovery is desired. Add 0.1 volume of 1 M Na_2HPO_4 (or 1M Tris-HCl) solution to the recovered supernatant to neutralize the pH.

Regeneration of MPG® Protein G:

(The following protocol has been developed using 10% serum. More concentrated samples may require additional washing steps.)

1. Resuspend the MPG® Protein G particles in 0.5 ml Elution Buffer and mix well. Incubate at room temperature for 5-10 minutes with mixing on a low speed rotator. Magnetically separate and remove the supernatant.

2. Resuspend the particles in 0.5 ml PBS and mix well. Magnetically separate and remove the supernatant.

3. Resuspend the particles in 0.5 ml Storage Buffer and mix well. Magnetically separate and remove the supernatant.

4. Add Storage Buffer to give a 1% suspension and mix well. Store at 4ºC.

NOTE: *If you have been using the particles with strongly binding immunoglobulin from one or several different species, or with samples containing high concentrations of protein (>10 mg/ml), it may be beneficial to substitute 20% acetic acid for Elution buffer in step 1 and repeat step two twice. Also, if you have used the particles with samples containing high concentrations of lipid, a PBS wash containing 10% methanol or 0.05% Tween 20 inserted between steps 1 and 2 will help remove these contaminants.*

References

1. McGuire, J. (1989) ABL 7:28.
2. Erntell, M. et al. (1988) Molecular Immunology 25:121.
3. Fahnestock, S.R. et al. (1986) J. Bacteriol. 167:870.
4. Akerstrom, B and Bjorck, L. (1986) J. Biol. Chem. 261(22):10240.

*This product contains **sodium azide** as a preservative. Sodium azide may react with lead and copper plumbing to form explosive metal azides. On disposal of material, flush with large volumes of water to prevent azide accumulation.*

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