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Products for Biotechnology
With Magnetic Porous Glass (MPG®)

Protocol No.: 57.105
Product: MPG® Protein A (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
MPGPA0501	MPG® Protein A, 5 µm, 500 Å pore diameter	1 ml (10 mg)
MPGPA0505		5 ml (50 mg)
MPGPA0510		10 ml (100 mg)

Principle and Applications

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

Recombinant protein A has a molecular mass of 42,000 daltons but migrates on SDS PAGE with an apparent mass of ~45,000 daltons. Although protein A has five immunoglobulin F(c) binding sites only two of these are used at one time(1). This allows maximum biological activity of the antibody by orienting its constant region towards the particle while giving full access to antigen. Immunoglobulin binding is very strong (K_a=10^9) (2) yet reversible at low pH.

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 A particles is between 40 - 76 µg of rabbit IgG. See product specification sheet for lot specific information.

Guidelines for Optimal Use

Affinity for different immunoglobulins by protein A varies as shown in Table 1. Most sera can be used without concern. However, polyclonal immunoglobulin from chicken, goat, rat and sheep and monoclonal mouse IgG_1, rat IgG_1, IgG_{2a} and IgG_{2b}, and human IgG_3 will either fail to bind or give non-quantitative binding. In these situations we recommend using MPG® Protein G or one of our MPG® Secondary Antibody Products.

Binding of immunoglobulin to protein A is impacted by **buffer conditions** (4). The optimal binding pH is generally near 8.0. Borate, carbonate, glycine-NaOH, HEPES, phosphate, and Tris are among the buffers that have been used. The typical salt concentration is physiological saline. Higher (molar) concentrations of salt have been shown to improve binding of monoclonals and other weak binding species, but at the same time increases nonspecific binding of proteins. When a high salt concentration is required, MPG® Protein A will perform without the need for surfactants and without concern for clumping or adhering to hydrophobic surfaces.

Other factors affecting the binding reaction include **temperature**, **concentration** of both particles and immunoglobulin and **incubation time**. These factors are application specific. Although temperatures ranging from 4°C (especially for some monoclonal antibodies and labile samples) to 37°C (certain immunoassays) have been used, most binding reactions can be conducted at room temperature. The optimal concentrations of

immunoglobulin and particles and incubation time should be optimized empirically by the researcher. As a starting point, using an excess of 1 mg MPG® Protein A particles at room temperature in 1 ml PBS, both rabbit IgG and goat IgG conjugated to HRP can be completely cleared (>96%) within 5 minutes at concentrations as low as 1 µg/ml. Using lower concentrations of particles and/or immunoglobulin, particularly if complexed to antigen may require overnight or longer times for complete binding.

Table 1. Binding specificity of protein A for immunoglobulins from different species.

SPECIES	SUBCLASS	AFFINITY OF PROTEIN A[a]
Bovine	IgG1	Weak
	IgG2	Strong
Chicken	IgG	None
Dog	IgG	Strong
Donkey	IgG	(49-55)
Goat	IgG	(31-34)
	IgG1	Weak
	IgG2	Strong
	IgM	None
Guinea Pig	IgG1	Strong
	IgG2	Strong
Horse	IgG	Weak
Human	IgG	(53-54)
	IgG1	Strong
	IgG2	Strong
	IgG3	Weak
	IgG4	Strong
	IgA2	Weak
	IgM	Weak
Mouse	IgG	(34-57)
	IgG1	Weak (11-19)
	IgG2a	Strong
	IgG2b	Strong
	IgG3	Strong
	IgM	None
Pig	IgG	Strong
Rabbit	IgG	Strong (55-76)
	IgM	None
Rat	IgG	(35-37)
	IgG1	Weak
	IgG2a	None
	IgG2b	None
	IgG2c	Strong
	IgM	None
Sheep	IgG	(43-55)
	IgG1	Weak
	IgG2	Strong
	IgM	None

[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 A using either purified IgG in PBS or 10% serum in 0.15 M NaCl, 1 M Glycine, pH 8.6.

Recovery of immunoglobulin (or immunoglobulin-antigen complex) from the particles can be accomplished at acid pH. The acidity needed depends on the species and subclass. Weaker binding immunoglobulins (e.g., sheep) can be eluted off in the pH 4 to pH 6 range, while stronger binding species (e.g., rabbit) require pH 3 to 3.5. Essentially all immunoglobulin can be removed at pH 3.0. Binding activity 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, acetate, phosphate or other biological buffers for elution. We do not recommend using acidic citrate buffers with MPG® Protein A. Immunoglobulin can be recovered in concentrated form with MPG® Protein A. Using 10-50 µl of 0.1 M glycine-HCl, pH 2.5 per mg particles will give 75% recovery of captured immunoglobulin. An additional wash with the same volume of buffer will give an additional 10% recovery. Elution buffers of higher pH and/or lower ionic strength may give somewhat different results due to the buffering capacity of the particles. All the immunoglobulin will be recovered in 0.45 ml of 0.1M glycine, pH 2.5.

With proper use and storage, MPG® Protein A particles can be reused. Using 10% normal rabbit serum as an immunoglobulin source, the particles can be regenerated up to 12 times while still providing greater than 90% of initial binding capacity. SDS-PAGE analysis of immunoglobulin recovered using regenerated particles shows no decrease in purity.

Example Procedure

Materials: *MPG® Protein A 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)
10 N Sodium Hydroxide (NaOH)
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)
Methanol
Sodium Azide (NaN_3)
Sodium Phosphate, Dibasic (Na_2HPO_4)
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.
Binding/Wash Buffer I (0.15 M NaCl, 1 M Glycine-NaOH, pH8.6)	Dissolve 8.7 g NaCl and 75 g Glycine in 700 ml dH_2O. Adjust pH to 8.6 with NaOH and adjust volume to 1000 ml with H_2O.
Binding/Wash Buffer II (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/Regeneration Buffer I (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.)
Regeneration Buffer II (10% Methanol in PBS)	Add 10 ml Methanol to 90 ml PBS.
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 an example. You will need to substitute and/or modify buffers as your application demands (discussed in the previous section).*

Preparation of MPG® Protein A:

- *The following protocol was developed for use with 1-5 mg of particles. For quantities outside this range the protocol can be scaled accordingly.*

- *Depending on your application this preliminary cycling procedure may be necessary in whole or part to give maximum immunoglobulin purity. It will remove various additives including BSA and anti-microbials, remove any protein A leached during prolonged storage, and concentrates the particles*.

1. Vortex the MPG® Protein A 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 A particles in 0.5 ml PBS and mix well. Magnetically separate and aspirate the supernatant. Repeat this step one more time.

3. Resuspend the MPG® Protein A particles in 0.5 ml Regeneration Buffer I and mix well. Magnetically separate and aspirate the supernatant.

4. Resuspend the MPG® Protein A particles in 0.5 ml Binding/Wash Buffer I or II depending on sample (see NOTE in the section below) and mix well. Magnetically separate and aspirate the supernatant.

Binding of Immunoglobulin to MPG® Protein A:

NOTE: *The following protocol has been developed using 10% serum. More concentrated samples and samples containing particulates will require additional washing steps. Use Binding/Wash Buffer I for weak binding immunoglobulins. Use Binding/Wash Buffer I or II for immunoglobulins with high affinity. If elution of immunoglobulin in a minimal amount of buffer is desired, a final wash with Binding Wash Buffer II (PBS) is advisable to remove traces of the 1 M Glycine in Binding/Wash Buffer I.*

1. Resuspend the particles in an immunoglobulin solution prepared in the appropriate Binding/Wash Buffer (see note above. 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 aspirate the supernatant.

3. Wash by adding 0.5 ml of the same Binding/Wash Buffer used in Step 1 to the immunoglobulin-bound MPG® Protein A particles and mix well. Magnetically separate and aspirate the supernatant. Repeat one more time.

4. For the final wash, add 0.5 ml of Binding/Wash Buffer II (Binding/Wash Buffer I can be substituted if used for the binding step and the elution volume is large, see NOTE above) to the immunoglobulin-bound MPG® Protein A particles and mix well. Magnetically separate and aspirate the supernatant. Repeat one more time.

Elution of Immunoglobulin from MPG® Protein A:

Recover immunoglobulin by suspending the complex in 50 µl Elution Buffer (as little as 10 µl may be used, see *Guidelines for Optimal Use Section*) for 5 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 Solution to the recovered supernatant to neutralize the pH.

Regeneration of MPG® Protein A:

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

1. Resuspend the MPG® Protein A particles in 0.5 ml Regeneration Buffer I and mix well. Magnetically separate and aspirate the supernatant.

2. Resuspend the particles in 0.5 ml Regeneration Buffer II and mix well. Magnetically separate and aspirate the supernatant.

3. Resuspend the particles in 0.5 ml Binding/Washing Buffer II and mix well. Magnetically separate and aspirate the supernatant.

4. Resuspend the particles in 0.5 ml Storage Buffer and mix well. Magnetically separate and aspirate the supernatant.

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

References

1. Surolia, A., Pain, D., and Islam Khan, M. (1982) TIBS Feb, 74-76.
2. Akerstrom, B., and Bjorck, L. J. Biol. Chem. (1986) 261(22):10240-10247.
3. Godfrey, M.A.J. (1997). In Affinity Separations, A Practical Approach (ed. Paul Matejtschuk), p. 174. IRL Press, Oxford.
4. US Patent Number US4704366.

*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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