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Products for Biotechnology
With Magnetic Porous Glass (MPG®)

Protocol No.: **47.4**
Product: **MPG® Goat anti-Mouse IgG (H&L)** (10 mg/ml, 4-6 x 10^7 particles/ml)
Procedure: **Binding Mouse IgG**
Storage: **2-8ºC, Do Not Freeze.**

PRODUCT NUMBER	DESCRIPTION	VOLUME
G-AB11020	MPG® Goat anti-Mouse IgG (H&L)	2 ml (20 mg)
G-AB11100		10 ml (100 mg)

MPG® Goat anti-Mouse IgG is a suspension of spheroidal totally porous borosilicate glass particles embedded with superparamagnetic iron oxide to which affinity purified Goat anti-Mouse IgG antibody has been covalently attached. This Goat secondary antibody binds both the heavy and light chains of all of the Mouse IgG subclasses (IgG_1, IgG_{2a}, IgG_{2b}, and IgG_3). Human cross reactivity is minimal. MPG® Secondary Antibody particles eliminate centrifugation, allow easier scale-up and automation, and give flexibility in assay configuration. These particles can be used as a secondary antibody in single or automated enzyme and radio immunoassays utilizing a Mouse IgG monoclonal or polyclonal antibody. They are highly recommended for separating unbound labeled tracer. These particles are excellent in small-scale immunoaffinity purifications or immunoprecipitation reactions for gel or other physical analyses. Use of magnetic particles not only reduces assay time, but as a result increases performance. These particles should be used in conjunction with a magnetic particle separator to achieve purification in minutes. The binding capacity of this antibody may vary among IgG isotypes and analyte bound IgG. In these instances, the researcher may have to determine actual binding capacities for their particular system. Check the accompanying certificate of analysis for lot specific information. The optimal dilution for specific applications should be determined by the researcher.

Store the vial at 2-8° C. 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. Do not freeze or dry, as loss of binding activity and aggregation will occur. Dilute only prior to immediate use. Expiration date is six (6) months from date of opening product.

General Procedure

Materials: *MPG® Goat anti-Mouse IgG 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)
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)
Sodium Phosphate, Dibasic (Na_2HPO_4)
Tween 20
1.5 ml Microcentrifuge Tubes
Magnetic Particle Separator, Prod. No. MPS0301 or MPS0001
Low Speed Rotator
Pipettes and Pipette Tips
Vortex Mixer
Primary Antibody

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.
Binding Buffer (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 to7.4 with 2 N HCl and adjust volume to 1000 ml with dH_2O.
Washing Buffer (PBS, 0.05% Tween 20)	Mix 0.25 gm Tween 20 in 500 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 Binding Buffer.
Elution Buffer (0.1M Glycine-HCl, pH 2.5)	Dissolve 7.5 g Glycine in 500 ml dH_2O. Adjust pH to 2.5 with HCl and adjust volume to 1000 ml with H_2O.

**If the desired antibody cross-reacts with BSA, serum albumin of other species should be used in Storage Buffer.*

NOTE: These buffer formulations are only intended as a general guideline. Feel free to substitute and/or modify buffers as your application demands.

Preparation of MPG® Goat anti-Mouse IgG Particles:

Depending on your application a **preliminary wash step** using an appropriate buffer may be necessary. Washing will remove various additives including EDTA and anti-microbials, and concentrates the particles.

NOTE: For cell separations and further culturing, it is recommended that the particles be washed 5X in sterile medium prior to use.

1. Vortex the MPG® Goat anti-Mouse IgG 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® Goat anti-Mouse IgG particles in Binding Buffer to original starting concentration (or more) and mix well. Magnetically separate and aspirate the supernatant. Repeat this step two more times.

Attachment of an Antibody to MPG® Goat anti-Mouse IgG Particles:

(The volumes and concentrations can be adjusted to suit particular applications.)

1. Resuspend the particles to give 0.5 mg solids/ml in a solution of Binding Buffer containing primary antibody. The antibody concentration should be determined empirically, but can be based on the binding capacity of the particles, as shown on the Certificate of Analysis.

2. Incubate at room temperature for 30 minutes with gentle mixing on low speed rotator.

3. Add Washing Buffer to the antibody-bound MPG® Goat anti-Mouse IgG particles to a concentration of 0.5 mg solids/ml and mix well. Magnetically separate and aspirate the supernatant. Repeat four more times. The antibody-bound MPG® Goat anti-Mouse IgG particles are now ready for use in your specific application.

Storage of Primary Antibody Coated Particles:

For storage, add 1 ml of Storage Buffer to the antibody-bound MPG® Goat anti-Mouse IgG particles and mix well. Magnetically separate and aspirate the supernatant. Resuspend the antibody-bound MPG® Goat anti-Mouse IgG particles in 1 ml of Storage Buffer to the desired storage concentration (often 0.5 mg/ml) and store at 4ºC.

Elution of Primary Antibody from MPG® Goat anti-Mouse IgG Particles:

Break antibody/antigen interaction by suspending the complex in Elution Buffer for 5 minutes, with gentle mixing, at room temperature. Any convenient volume can be used, but the minimum should be 10-15 µl in order to adequately cover the particles and inside surface of the microfuge tube. Magnetic particles can then be separated and the supernatant, containing analyte, can be used in your application. Add 0.1 volume of 1 M Na_2HPO_4 to the recovered supernatant to neutralize the pH, if your analyte is acid labile.

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