

Product No. B-6649

Lot 086H8950

Anti-Mouse IgG (whole molecule)

Biotin Conjugate

Antibody developed in Goat

Affinity Isolated Antigen Specific Antibody

Adsorbed with Human Serum Proteins

Antiserum is developed in goat using purified mouse IgG as the immunogen. Affinity isolated antigen specific antibody is obtained from goat anti-mouse IgG antiserum by immunospecific purification which removes essentially all goat serum proteins, including immunoglobulins, that do not specifically bind to mouse IgG. The antibody preparation is solid phase adsorbed with human serum proteins to ensure minimal cross reactivity in tissue or cell preparations. Goat anti-Mouse IgG is conjugated to N-hydroxysuccinimidobiotin (Sigma Product No. H-1759). The conjugate is provided as a solution in 0.01M phosphate buffered saline, pH 7.4, containing 1% BSA and 0.1% sodium azide (see MSDS)* as a preservative.

Specificity

Specificity of the anti-mouse IgG antibodies is determined by immunoelectrophoresis (IEP) versus normal mouse serum and mouse IgG. Cross-reactivity of the antibody preparation is determined by Ouchterlony Double Diffusion (ODD). The antibody shows no reactivity with human serum proteins.

Identity and Purity

Identity and purity of the antibody is established by immunoelectrophoresis, prior to conjugation. Electrophoresis of the antibody preparation followed by diffusion versus anti-goat IgG and anti-goat whole serum results in single arcs of precipitation.

Antibody Content

The product is provided with a specific antibody content of 0.3 mg/ml (prior to the addition of BSA).

Working Dilution: 1:20,000 (minimum)

Working dilution is defined as the dilution of conjugate that give a change in absorbance of 1.0 at 492 nm after 30 minutes of substrate conversion at 25°C (Voller, et al., and Guedson et al.)^{2,3}. Microtiter plates are coated with purified mouse IgG at a concentration of 200 ng/ml in 0.05 M carbonate/bicarbonate buffer pH 9.6 (Carbonate/Bicarbonate Buffer Capsules are available as Sigma Product No. C-3041). Following incubation with the biotinylated antibody, a solution of Avidin-Horseradish Peroxidase (Sigma Product No. A-3151, diluted in 0.01 M phosphate buffered saline, pH 7.4, containing 0.05% Tween 20 and 0.5% BSA) is added.

Substrate: 0.04% o-Phenylenediamine Dihydrochloride* (OPD, Sigma Product No. P-8412), and 0.012% Hydrogen Peroxide* (H₂O₂, Sigma Product No. H-1009) in phosphate-citrate buffer, pH 5.0 [25.7 ml 0.2 M dibasic sodium phosphate (Sigma Product No. S-876), 24.3 ml 0.1 M citric acid (Sigma Product No. C-7129) and 50 ml deionized water].

*Add immediately before use.

In order to obtain best results in different preparations, it is recommended that each individual user determine their optimum working dilution by titration assay.

Storage

For continuous use, store at 2-8°C. For extended storage, the solution may be frozen in working aliquots. Repeated freezing and thawing is **not** recommended. Storage in "frost-free" freezers is **not** recommended. If slight turbidity occurs upon prolonged storage, clarify the solution by centrifugation before use.

References

1. Bayer, E., et al., Methods in Enzymology, **62**, 308 (1979).
2. Voller, A., et al., Bulletin WHO, **53**, 55 (1976).
3. Guedson, J., et al., J. Histochem. and Cytochem., **27**, 1131 (1979).

*Due to the sodium azide content a material safety data sheet (MSDS) for this product has been sent to the attention of the safety officer of your institution. Consult the MSDS for information regarding hazards and safe handling practices.