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Product Information

Anti-Mouse IgG (whole molecule)—Alkaline Phosphatase

produced in goat, affinity isolated antibody adsorbed with human serum proteins

Catalog Number A3688

Product Description

Anti-Mouse IgG (whole molecule) is produced in goat using purified mouse IgG as the immunogen. Affinity isolated antibody is obtained from anti-mouse IgG antiserum by immunospecific purification which removes essentially all goat serum proteins, including immunoglobulins, which 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. Anti-Mouse IgG is conjugated to alkaline phosphatase by protein cross-linking with 0.2% glutaraldehyde. 1.

Specificity of the antiserum is determined by immunoelectrophoresis (IEP) and Ouchterlony Double Diffusion (ODD) assays, prior to conjugation. By IEP, the antiserum reacts specifically with normal mouse serum and mouse IgG. By ODD, the antiserum is found to be reactive with mouse IgG1, IgG2a, IgG2b, IgG3, IgA and IgM. The conjugate shows no reactivity with human serum proteins by ELISA.

Reagent

Supplied as a solution in 0.05 M Tris buffer, pH 8.0, containing 1% BSA, 1 mM MgCl₂, 50% glycerol, and 15 mM sodium azide as a preservative.

Precautions and Disclaimer

This product is for R&D use only, not for drug, household, or other uses. Please consult the Material Safety Data Sheet for information regarding hazards and safe handling practices.

Storage/Stability

Store at 2-8 °C.

Product Profile

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

Titers

Direct ELISA: minimum 1:30,000

Titer is defined as the dilution of conjugate sufficient to give a change in absorbance of 1.0 at 405 nm after 30 minutes of substrate conversion at 25 $^{\circ}$ C. Microtiter plates are coated with purified mouse IgG at a concentration of 5 μ g/ml in 0.05 M carbonate-bicarbonate buffer, pH 9.6. Carbonate-Bicarbonate Buffer capsules are available as Catalog Number C3041.

Substrate: *p*-Nitrophenyl Phosphate (pNPP), Catalog Number N2765, 1.0 mg/mL in 10% diethanolamine buffer, pH 9.8, containing 0.5 mM MgCl₂.

<u>Dot Blot</u>: minimum 1:30,000 Diluted conjugate detects up to 20 ng mouse IgG bound to nitrocellulose.

Substrate: 5-Bromo-4-chloro-3-indolyl Phosphate/Nitroblue Tetrazolium (BCIP/NBT), SIGMA*FAST*[™] Tablets, Catalog Number B5655.

Immunohistology: minimum 1:50

Determined by an indirect assay using formalin-fixed, paraffin-embedded sections of human tonsil and Monoclonal Anti-Human IgG, Catalog Number I6760, as the primary antibody.

Substrate: Fast Red TR/AS-MX Napthol Phosphate³ SIGMA*FAST* Tablets, Catalog Nos. F4523 or F4648.

Western Blotting: 1:30,000

Mouse IgG was detected directly using 10 μ g protein under reducing conditions on an SDS-PAGE gradient (4-20%) gel. The protein was transferred to nitrocellulose, blocked with 5% BSA in 0.05 M Tris and then incubated with the conjugate.

Substrate: 5-Bromo-4-chloro-3-indolyl Phosphate/Nitroblue Tetrazolium (BCIP/NBT), SIGMA*FAST* Tablets, Catalog Number B5655.

Note: Working dilutions should be determined by titration assays. Due to differences in assay systems, these titers may not reflect the user's actual working dilution.

References

- 1. Avrameas, S., *Immunochemistry*, **6**, 43, (1969).
- 2. Voller, A., et al., *Bull. World Health Organ.*, **53**, 55 (1976).
- 3. Pluzek, K., and Ramlau, R., Alkaline Phosphatase Labeled Reagents, In: Vol. 1 *CRC Handbook of Immunoblotting of Proteins*, Bjerrum O., and Heegaard, N., (Eds.), p. 177CRC Press Inc., Boca Raton, FL, **1**,1988).

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