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Product Information

Anti-Mouse IgG (whole molecule)–Alkaline Phosphatase
produced in rabbit, affinity isolated antibody

Catalog Number **A4312**

Product Description

Antiserum is produced in rabbit using purified mouse IgG as the immunogen. Affinity isolated antibody is obtained from rabbit antiserum by immunospecific purification which removes essentially all rabbit serum proteins, including immunoglobulins, which do not specifically bind to mouse IgG. 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.

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

Reagent

Provided as a solution in 0.05 M Tris buffer, pH 8.0, containing 1% BSA, 1 mM $MgCl_2$, 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

Store at 2-8 °C.

Product Profile

Direct ELISA: minimum titer 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 °C.[2]
Microtiter plates are coated with purified mouse IgG at a concentration of 5 µg/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 $MgCl_2$.

Immunoblotting: working dilution of 1:30,000 is determined using an immunoblot assay detecting ß-Actin in total cell extract of HeLa cells (5-10 ug/well)

Immunohistology: minimum dilution 1:50 determined by an indirect assay using formalin-fixed, paraffin-embedded sections of human tonsil and Monoclonal Anti-Human IgM, Catalog Number I6385, as the primary antibody.

Substrate: Fast Red TR/AS-MX Napthol Phosphate[3] SIGMA*FAST*™ Tablets, Catalog Nos. F4523 or F4648.

Western Blotting: minimum dilution 1:30,000
Mouse IgG was detected directly using 10 µg protein. Reducing conditions on an SDS-PAGE gradient (4-20%) gel were used. 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, V., *Immunochemistry*, **6**, 43, (1969).
2. Voller, A., et al., *Bulletin WHO*, **53**, 55 (1976).
3. Pluzek, K. and Ramlau, R., Alkaline Phosphatase Labeled Reagents, In: CRC Handbook of Immunoblotting of Proteins, Vol. 1: Technical Descriptions, Bjerrum O. and Heegaard, N., (Eds.), CRC Press Inc., Boca Raton, FL, p. 177, 1988.

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