

Product Information

Anti-Mouse IgG (whole molecule)–Alkaline Phosphatase

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

Catalog Number **A9316**

Product Description

Antiserum 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 rat 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. Cross reactivity of the antibody-conjugate is determined by ELISA. The conjugate shows no reactivity with rat IgG or rat serum proteins.

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

Reagent

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

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 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 MgCl₂.

Dot Blot: minimum 1:30,000

Diluted conjugate detects < 20 ng mouse IgG bound to nitrocellulose.

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

Immunohistochemistry: 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 Naphthol Phosphate³ SIGMAFAST Tablets, Catalog Nos. F4523 or F4648.

Western Blotting: minimum 1:30,000

Mouse IgG was detected directly using 10 µg protein. Non-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), SIGMAFAST 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 amlau, R., Alkaline Phosphatase Labeled Reagents, In: *CRC Handbook of Immunoblotting of Proteins*, Bjerrum O., and Heegaard, N., (Eds.), CRC Press Inc., Boca Raton, FL, **1**, p. 177, 1988.

SIGMAFAST is a trademark of Sigma-Aldrich Biotechnology LP and Sigma-Aldrich Co.

TD,KAA,PHC 06/01/09