

Product Information

Anti-Rabbit IgG (whole molecule)- Alkaline Phosphatase

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

Catalog Number **A3812**

Product Description

Anti-rabbit IgG (whole molecule) is produced in goat using purified rabbit IgG as the immunogen. Antibody is isolated from anti-rabbit IgG antiserum by immunospecific purification which removes essentially all goat serum proteins, including immunoglobulins that do not specifically bind to rabbit IgG. The antibody preparation is solid phase adsorbed with human serum proteins to ensure minimal cross reactivity in tissue or cell preparations. Anti-Rabbit IgG is conjugated to alkaline phosphatase by protein cross linking with 0.2% glutaraldehyde.¹

Specificity of the antiserum is determined by immunoelectrophoresis prior to conjugation, versus normal rabbit serum and rabbit IgG.

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

Supplied as a solution in 0.05 M Tris buffer, pH 8.0, containing 1% BSA, 1mM MgCl₂, 50% glycerol and 10 mM glycine with 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 titer of 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 rabbit 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 titer 1:30,000

Diluted conjugate detects ≤20 ng rabbit IgG bound to nitrocellulose.

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

Immunohistology: minimum titer of 1:50

Determined by an indirect assay using formalin-fixed, paraffin-embedded sections of human tonsil and Anti-Human IgG, Catalog Number I2011, as the primary antibody.

Substrate: Fast Red TR/AS-MX Naphthol Phosphate³ SIGMAFAST Tablets, Catalog Nos. F4523 or F4648.

Immunoblotting: minimum titer 1:30,000

Rabbit IgG was detected directly using 10 µg protein under reducing conditions on a 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. Human IgG and normal human serum were also run (10 µg). No reaction with conjugate was observed.

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., *Bull. World Health Organ.*, **53**, 55 (1976).
3. Pluzek, K. J. and R. Ramlau, Alkaline Phosphatase Labeled Reagents, in CRC Handbook of Immunoblotting of Proteins, O. J. Bjerrum and N.H.H. Heegaard, Eds., CRC Press Inc., Boca Raton, FL, **1**, p. 177, 1988.

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