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Product Information

Anti-Human IgG (γ-Chain Specific) $F(ab')_2$ Fragment–Alkaline Phosphatase
antibody produced in goat, affinity isolated antibody

Catalog Number **A3312**

Product Description

Anti-Human IgG is produced in goat using purified human IgG as the immunogen. The $F(ab')_2$ fragment of the antibody is obtained from pepsin digested antiserum by immunospecific methods of purification. Affinity isolation removes essentially all goat serum proteins, including immunoglobulins which do not specifically bind to the γ-chain of human IgG. Anti-Human IgG is conjugated to alkaline phosphatase by protein cross linking with 0.2% glutaraldehyde.[1]

Specificity of Anti-Human IgG-Alkaline Phosphatase is determined by Enzyme Linked Immunosorbent Assay (ELISA). The conjugate is specific for human IgG when tested against human IgA, IgG, IgM, and Bence Jones Kappa and Lambda myeloma proteins.

Identity and purity of the antibody is established by immunoelectrophoresis (IEP), prior to conjugation. Electrophoresis of the antibody preparation followed by diffusion against anti-goat IgG and anti-goat whole serum results in single arcs of precipitation. The antibody preparation is found to consist only of the $F(ab')_2$ fragment of goat IgG as determined by SDS-PAGE. No contamination with goat IgG whole molecule is observed.

Reagent

Supplied as a solution in 0.05 M Tris buffer, pH 8.0, containing 1% BSA, 1 mM $MgCl_2$, 50% glycerol, 10 mM glycine, 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

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.[1] Microtiter plates are coated with purified human 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 Cat. No. C3041.

Substrate: *p*-Nitrophenyl Phosphate (pNPP), Cat. No. N2765, 1.0 mg/ml in 10% diethanolamine buffer, pH 9.8, containing 0.5 mM $MgCl_2$.

Dot Blot: Minimum 1:30,000
Diluted conjugate detects a minimum of 20 ng human IgG bound to nitrocellulose.

Substrate: 5-bromo-4-chloro-3-indolyl Phosphate/-Nitroblue Tetrazolium (BCIP/NBT), SIGMA*FAST*™ Tablets, Cat. No. B5655

Immunohistology: Minimum 1:50
Determined by a direct assay using formalin-fixed, paraffin-embedded sections of human tonsil.

Substrate: Fast Red TR/AS-MX Napthol Phosphate[3], SIGMA*FAST* Tablets, Cat. No. F4523 or F4648.

Western Blotting: Minimum 1:30,000
Human IgG was detected directly using 10 µg protein per lane. Reducing conditions on an SDS-PAGE gradient (4-20%) gel were used. The protein was transferred to nitrocellulose, blocked with 0.5% 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, Cat. No. 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. J., and Ramlau, R., *Alkaline Phosphatase Labeled Reagents, in CRC Handbook of Immunoblotting of Proteins*, Bjerrum, O. J., and Heegaard, N. H. H., eds., p. 177, (CRC Press Inc., Boca Raton, FL, 1988).

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