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Product Information

Anti-Human IgM (μ-chain specific)–Alkaline Phosphatase
produced in goat, affinity isolated antibody

Catalog Number **A3437**

Product Description

Antibody is prepared from anti-human IgM antiserum by immunospecific purification to remove essentially all goat serum protein, including immunoglobulins, which do not bind specifically to the μ-chain of human IgM. Conjugation of the antibody preparation to alkaline phosphatase is accomplished by protein cross-linking with 0.2% glutaraldehyde.[1]

Specificity of Anti-Human IgM-Alkaline Phosphatase is determined by Enzyme Linked Immunosorbent Assay (ELISA). The conjugate is specific for human IgM when tested against human IgA, IgG, IgM, Bence Jones kappa, and lambda myeloma proteins.[2]

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.

Reagents

Supplied 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: a minimum titer of 1:30,000 is determined. 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 human IgM 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 $MgCl_2$.

Dot Blot: a minimum titer of 1:30,000 is determined. Diluted conjugate detects ≤ 20 ng human IgM bound to nitrocellulose.

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

Direct Immunohistochemistry: a minimum titer of 1:50 is 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, Catalog Nos. F4523 or F4648.

Immunoblotting: a minimum titer of 1:30,000 is determined. Human IgM was detected directly using 10 μg per lane. Reducing conditions on an SDS-PAGE gradient (4-20%) gel were used. The protein was transferred to nitrocellulose, blocked with 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., *Bull. World Health Organ*., **53**, 55 (1976).
3. Pluzek, K., and Ramlau, 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.

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