ANTI-HUMAN IgM (μ-CHAIN SPECIFIC)
ALKALINE PHOSPHATASE CONJUGATE
Antibody Developed in Goat

Product No. **A1067**

Lot No. 117H9245

$F(ab')_2$ Fragment of Affinity Isolated Antigen Specific AntibodyAnti-Human IgM is developed in goat using purified human IgM 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 μ-chain of human IgM. Goat anti-human IgM is conjugated to Alkaline Phosphatase by protein cross linking with 0.2% glutaraldehyde.[1] The conjugate is provided as a solution in 0.05 M Tris buffer, pH 8.0, containing 1% BSA, 1 mM $MgCl_2$, 50% glycerol, and 0.1% sodium azide (see MSDS)* as a preservative.

Specificity
Specificity of the Alkaline Phosphatase Conjugated Goat Anti-Human IgM 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.

Identity and Purity
Identity and purity of the antibody is established by immunoelectrophoresis (IEP), prior to conjugation. Electrophoresis of the antibody preparation followed by diffusion versus 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-Polyacrylamide Gel Electrophoresis (PAGE). No contamination with goat IgG whole molecule is observed.

Titers
1. ELISA: 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 (Voller, et al.[1]). 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 Sigma Product No. C3041).
Substrate: *p*-Nitrophenyl phosphate (pNPP, Sigma Product No. N2765), 1.0 mg/ml in 10% diethanolamine buffer, pH 9.8, containing 0.5 mM $MgCl_2$.

2. Dot Blot: 1:30,000
Diluted conjugate detects 4 ng human IgM bound to nitrocellulose.
Substrate: 5-Bromo-4-chloro-3-indolyl Phosphate/ Nitroblue Tetrazolium (BCIP/NBT, SIGMA *FAST*™ Tablets, B5655).

3. Immunohistology: 1:100
Determined by a direct assay using formalin-fixed, paraffin-embedded sections of human tonsil.
Substrate: Fast Red TR/AS-MX Naphthol Phosphate[3] (SIGMA *FAST*™ Tablets F4523 or F4648).

4. Western Blotting: 1:30,000
Human IgM 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 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, B5655).

Working Dilutions
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, Bjerrum O., and Heegaard, N., (Eds.), CRC Press Inc., Boca Raton, FL, **1**, p. 177, 1988.

Storage
Store at 2-8°C. **Do Not Freeze**

*Due to the sodium azide content a material safety data sheet (MSDS) for this product has been sent to the attention of the safety officer of your institution. Consult the MSDS for information regarding hazards and safe handling practices.

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