ANTI-HUMAN IgA (α-CHAIN SPECIFIC)
Alkaline Phosphatase Conjugate
Antibody developed in Goat
Affinity Isolated Antigen Specific Antibody

Product No. **A3063**
Lot 067H8970

Antiserum is developed in goat using purified human IgA as the immunogen. Affinity isolated antigen specific antibody is obtained from goat anti-human IgA antiserum by immunospecific purification which removes essentially all serum proteins, including immunoglobulins, which do not specifically bind to the α-chain of human IgA. Conjugation of the antibody preparation to Alkaline Phosphatase is accomplished by protein cross-linking with 0.2% glutaraldehyde.[1] This product is provided as a solution in 0.05 M Tris buffer, pH 8.0, containing 1% BSA, 50% glycerol, 1 mM $MgCl_2$ and 0.1% sodium azide (see MSDS)* as a preservative.

Specificity
Specificity of the Alkaline Phosphatase Conjugated Anti-Human IgA is determined by Enzyme Linked Immunosorbent Assay (ELISA). The conjugate is specific for human IgA 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.

Titers
1. 1:39,000 (Direct ELISA)
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 IgA 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 (*p*NPP, 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 8 ng human IgA bound to nitrocellulose.
Substrate: 5-Bromo-4-chloro-3-indolyl Phosphate/ Nitroblue Tetrazolium (BCIP/NBT, SIGMA *FAST*™ Tablets, B5655).

3. Immunohistology: 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 F4523 or F4648).

4. Western Blotting: 1:30,000
Human IgA was detected directly using 20 µg protein per lane under reducing conditions on a PAGE gradient (4-15%) gel. 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, 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 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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