Product No. B-9015
Lot 084H8958

Anti-Human Polyvalent Immunoglobulins (α, γ, and μ-chains specific) Biotin Conjugate

Affinity Isolated Antigen Specific Antibodies
Antibodies Developed in Goats

Individual antisera to human IgA, IgG and IgM are developed in goats using purified immunoglobulin heavy chains (α, γ, and μ) as the immunogens. Affinity isolated antigen specific antibodies are obtained from each antisera by immunospecific purification which removes essentially all goat serum proteins, including immunoglobulins, that do not specifically bind to the heavy chains. Each specific antibody is then conjugated to Sigma N-Hydroxysuccinimidobiotin (Sigma Product No. H-1759) by a modification of the method of Bayer, et al.[1] The product is prepared by combining the conjugated antibodies to ensure consistant activity for each heavy chain. The conjugate is provided as a solution in 0.01 M phosphate buffered saline, pH 7.4, containing 1% BSA with 0.1% sodium azide (see MSDS)* as a preservative.

Specificity

Specificity of the Biotin Conjugated Anti-Human Polyvalent Immunoglobulins is determined by Enzyme Linked Immunosorbent Assay (ELISA). The conjugate is specific for human IgA, IgG and 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.

Antibody Content

The product is provided with a specific antibody content of 0.81 mg/ml (prior to the addition of BSA).

Working Dilution: 1:7,000 (minimum, each immunoglobulin)

Working dilution is defined as the dilution of conjugate that gives a change in absorbance of 1.0 at 492nm after 30 minutes of substrate conversion at 25°C (Voller, et al. and Guedson et al.)[2,3]. Microtiter plates are coated individually with purified human IgA, IgG and IgM at concentrations of 200 ng/ml in 0.05M carbonate/bicarbonate buffer pH 9.6 (carbonate/bicarbonate buffer capsules are available as Sigma Product No. C-3041). Following incubation with the biotinylated antibody, a solution of Avidin-Horseradish Peroxidase (Sigma Product No. A-3151, diluted in 0.01 M phosphate buffered saline, pH 7.4, containing 0.05% Tween 20 and 0.5% BSA) is added.

Substrate: 0.04% *o*-Phenylenediamine Dihydrochloride** (OPD, Sigma Product No. P-8412), and 0.012% Hydrogen Peroxide** (H_2O_2, Sigma Product No. H-1009) in phosphate-citrate buffer, pH 5.0 [25.7 ml 0.2M dibasic sodium phosphate (Sigma Product No. S-0876), 24.3 ml 0.1M citric acid (Sigma Product No. C-7129) and 50 ml deionized water].

**Add immediately before use.

Storage

For continuous use, store at 0-5°C. For extended storage, the solution may be frozen in working aliquots. Repeated freezing and thawing is **not** recommended. Storage in "frost-free" freezers is **not** recommended. If slight turbidity occurs upon prolonged storage, clarify the solution by centrifugation before use.

References

1. Bayer, E.A., et al., Methods in Enzymology, **62**, 308 (1979).
2. Voller, A., et al., Bulletin WHO, **53**, 55 (1976).
3. Guedson, J.L., et al., J. Histochem. and Cytochem., **27**, 1131 (1979).

*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.