

Product No. B-2641**Lot 017H8940****Anti-Human IgM (μ -chain specific)
Biotin Conjugate**

Antibody developed in Goat

F(ab')₂ Fragment of Affinity Isolated Antigen Specific Antibody

Antiserum 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 Sigma N-Hydroxysuccinimido-biotin (Sigma Product No. H-1759) by a modification of the method of Bayer, et al.¹ The conjugate is provided as a solution in 0.01 M phosphate buffered saline, pH 7.4, containing 1% BSA with 15 mM sodium azide (see MSDS)* as a preservative.

Specificity

Specificity of the Biotin Conjugated 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.

Working Dilution: 1:20,000 (minimum)

Working dilution is defined as the dilution of conjugate that will give a change in absorbance of 1.0 at 492 nm after 30 minutes of substrate conversion at 25°C

(Voller, et al., and Guedson et al.)^{2,3}. Microtiter plates are coated with purified human IgM at a concentration of 200 ng/ml in 0.05 M 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** (H₂O₂, Sigma Product No. H-1009) in phosphate-citrate buffer, pH 5.0 [25.7 ml 0.2 M 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.

Antibody Content

The product is provided with a specific antibody content of 0.4 mg/ml (prior to the addition of BSA).

Storage

For continuous use, store at 2-8°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.

*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.