

**Product No. B-7024**  
**Anti-Goat IgG (whole molecule)**  
**Biotin Conjugate**  
Antibody Developed in Rabbit  
Affinity Isolated Antigen Specific Antibody  
Adsorbed with Human Serum Proteins

**Lot** 113H88151

Antiserum is developed in rabbit using purified goat IgG as the immunogen. Affinity isolated antigen specific antibody is obtained from rabbit anti-goat IgG antiserum by immunospecific purification which removes essentially all rabbit serum proteins, including immunoglobulins, that do not specifically bind to goat IgG. The antibody preparation is solid phase adsorbed with human serum proteins to ensure minimal cross reactivity in tissue or cell preparations. Rabbit anti-goat IgG is conjugated to Sigma N-Hydroxysuccinimidobiotin (Sigma Product No. H-1759) by a modification of the method of Bayer, et al.<sup>1</sup> 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-goat IgG is determined by Enzyme Linked Immunosorbent Assay (ELISA). Cross reactivity of the antibody-conjugate is determined by ELISA. The conjugate shows no reactivity with human serum 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-rabbit IgG and anti-rabbit whole serum results in single arcs of precipitation.

#### **Antibody Content**

The product is provided with a specific antibody content of 0.3 mg/ml (prior to the addition of BSA).

**Working Dilution:** 1:20,000

Working dilution is defined as the dilution of conjugate that gives 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.)<sup>2,3</sup>. Microtiter plates are coated purified goat IgG 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\*\* (H<sub>2</sub>O<sub>2</sub>, 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 0.1 M 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.

Sigma warrants that its products conform to the information contained in this and other Sigma publications. Purchaser must determine the suitability of the products for its particular use. See reverse side of invoice or packing slip for additional terms and conditions of sale.

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