

Product Information

ANTI-RABBIT IgG (WHOLE MOLECULE)

Biotin Conjugate

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

Product Number **B9140**

Product Description

Antiserum is developed in sheep using purified rabbit IgG as the immunoen. The F(ab)₂ fragment of the antibody is obtained from pepsin digested antiserum by immunospecific methods of purification. Affinity isolation removes essentially all sheep serum proteins, including immunoglobulins which do not specifically bind to rabbit IgG. Sheep anti-rabbit IgG is conjugated to Sigma N-Hydroxysuccinimidobiotin (Product No. H1759) by a modification of the method of Bayer, et al.¹ Specificity of the isolated F(abN)₂ fragment of antirabbit IgG is determined by immunoelectrophoresis of normal rabbit serum and rabbit IgG followed by diffusion, prior to conjugation.

Identity and purity of the antibody is established by immunoelectrophoresis (IEP), prior to conjugation. Electrophoresis of the antibody preparation followed by diffusion versus anti-sheep IgG and anti-sheep whole serum result in single arcs of precipitation. The antibody preparation is found to consist only of the F(ab)₂ fragment of sheep IgG as determined by SDS-Polyacrylamide Gel Electrophoresis (PAGE). No contamination with sheep IgG whole molecule is observed.

Reagents

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 as a preservative.

Precautions and Disclaimer

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.

Product Profile

The product is provided with a specific antibody content of 0.1-2.0 mg/ml (prior to the addition of BSA).

ELISA : Minimum 1:20,000

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 rabbit 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 Product No. C3041). Following incubation with the biotinylated antibody a solution of Avidin-Horseradish Peroxidase (Product No. A3151, 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, Product No. P8412), and 0.012% Hydrogen Peroxide** (H₂O₂, Product No. H1009) in phosphate-citrate buffer, pH 5.0 [25.7 ml 0.2 M dibasic sodium phosphate (Product No. S0876), 24.3 ml 0.1 M citric acid (Product No. C7129) and 50 ml deionized water].

**Add immediately before use.

Storage/Stability

For continuous use, store at 2-8 °C for up to one month. 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., et al., *Methods in Enzymology*, **62**, 308 (1979).
2. Voller, A., et al., *Bulletin WHO*, **53**, 55 (1976).
3. Guedson, J., et al., *J. Histochem. and Cytochem.*, **27**, 1131 (1979).

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