

Product No. B-7140
Anti-Bovine IgG (whole molecule)
Biotin Conjugate
Affinity Isolated Antigen Specific Antibody
Antibody Developed in Rabbit

Lot 051H8856

Antiserum is developed in rabbit using purified bovine IgG as the immunogen. Affinity isolated antigen specific antibody is obtained from rabbit anti-bovine IgG antiserum by immunospecific purification which removes essentially all rabbit serum proteins, including immunoglobulins, that do not specifically bind to bovine IgG. Rabbit anti-Bovine IgG is conjugated to Sigma N-Hydroxysuccinimidobiotin (Sigma Product No. H-1759) by a modification of the method of Bayer, et al.¹ The conjugate is provided as a solution in 0.01M phosphate buffered saline, pH 7.4, containing 1% BSA with 0.1% sodium azide (see MSDS)* as a preservative.

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 0.56 mg/ml (prior to the addition of BSA).

Working Dilution: 1:20,000 (minimum)

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 purified bovine IgG at

a concentration 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 0.5mg/ml solution of Avidin-Horseradish Peroxidase (Sigma Product No. A-3151, diluted 1:200 in 0.01M 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 0.2M 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**, 113 (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.

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