Technical Bulletin

Anti-Horse IgG (Whole Molecule)–Peroxidase Antibody Produced in Rabbit

Affinity isolated antibody, buffered aqueous solution

A6917

Product Description

Antiserum is developed in rabbit using purified horse IgG as the immunogen. Antibody is isolated from rabbit anti-horse IgG antiserum by immunospecific purification which removes essentially all rabbit serum proteins, including immunoglobulins that do not specifically bind to horse IgG. Rabbit anti-horse IgG is conjugated to Sigma Horseradish Peroxidase, Type VI (Cat. No. P8375) by a modification of the periodate method of Wilson and Nakane.[1]

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.

Reagents

The conjugate is provided as a solution in 0.01 M phosphate buffered saline, pH 7.4, containing 1% BSA with 0.05% MIT as a preservative.

Product Profile

Titer: Minimum 1:10,000 (Direct ELISA)

We are now reporting lot specific information as a titer by direct ELISA rather than as a working dilution. Titer is defined as the dilution of conjugate sufficient to give a change in absorbance of 1.0 at 450 nm after 30 minutes of substrate conversion at 25 °C.[2] Microtiter plates are coated with purified horse IgG at a concentration of 5 µg/mL in 0.05 M carbonate/bicarbonate buffer, pH 9.6 (Carbonate/Bicarbonate Buffer Capsules are available as Product No. C 3041). Substrate: *o*-Phenylenediamine dihydrochloride (OPD, Cat. No.P8287), 0.4 mg/mL in 0.05 M phosphate-citrate buffer, pH 5.0 containing 0.03% sodium perborate (Phosphate-Citrate Buffer Capsules with Sodium Perborate are available as Cat. No. P4922).

Working dilution should be determined by titration assay. Due to product improvement and changes in the assay procedure, we now list a lot specific titer by direct ELISA for this product. Due to differences in assay systems, this titer may not reflect the user's actual working dilution.

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.

References

1. Wilson, M., and Nakane, P., In: Immuno-fluorescence and Related Staining Techniques, Elsiever/North Holland BioMedical Press, Amsterdam, p. 215 (1978).
2. Voller, A., et al., Bulletin WHO, **53**: 55 (1976).

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