

Product No. A-2290
Lot 074H8980

Anti-Human IgG (γ -chain specific)
Peroxidase Conjugate

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

Anti-Human IgG is developed in goat using purified human IgG 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 γ -chain of human IgG. Goat anti-human IgG is conjugated to Sigma Horseradish Peroxidase, Type VI (Sigma Product No. P-8375) by a modification of the periodate method of Wilson and Nakane.¹ The conjugate is provided as a solution in 0.01M phosphate buffered saline, pH 7.4, containing 1% BSA with 0.01% thimerosal as a preservative.

Specificity

Specificity of the peroxidase conjugated anti-human IgG is determined by Enzyme Linked Immunosorbent Assay (ELISA). The conjugate is specific for human IgG 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.

Titer: 1:19,000 (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 450nm after 30 minutes of substrate conversion at 25°C.² Microtiter plates are coated with purified human IgG at a concen-

tration of 5 μ g/ml in 0.05 M carbonate/bicarbonate buffer, pH 9.6 (Carbonate/Bicarbonate Buffer Capsules are available as Sigma Product No. C-3041).

Substrate: *o*-Phenylenediamine dihydrochloride (OPD, Sigma Product No. P-8287), 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 Sigma Product No. P-4922).

Working Dilution

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.

References

1. Wilson, M.B., and P.K. Nakane, Immunofluorescence and Related Staining Techniques (Elsevier/North-Holland Biomedical Press, Amsterdam), p215 (1978).
2. Voller, A., et al., Bulletin WHO, **53**, 55 (1976).

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.

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