

## ProductInformation

### ANTI-HUMAN IgG (?-CHAIN SPECIFIC) PEROXIDASE CONJUGATE Antibody Developed in Goat IgG Fraction of Antiserum

Product No. **A8775**  
Lot 034H8808

Antiserum is developed in goat using purified human IgG as the immunogen. Whole antiserum is fractionated and then further purified by ion exchange chromatography to provide the IgG fraction of antiserum. This fraction is essentially free of other goat serum proteins. Goat anti-Human IgG is then conjugated to peroxidase by a modification of the periodate method of Wilson and Nakane.<sup>1</sup> The conjugate is provided as a solution in 0.01M phosphate buffered saline, pH 7.4, containing 1.0% BSA and 0.01% thimerosal as a preservative.

#### Specificity

Specificity for the ?-chain of human IgG is determined by Enzyme Linked Immunosorbent Assay (ELISA), Ouchterlony Double Diffusion (ODD), and immunoelectrophoresis (IEP). The antibody preparation is specific for human IgG when tested against purified human IgG, IgA, IgM, Bence Jones Kappa and Bence Jones Lambda myeloma proteins.

#### Identity and Purity

Identity and purity of the antibody is established by immunoelectrophoresis, prior to conjugation. Electrophoresis of the product followed by diffusion versus anti-goat IgG and anti-goat whole serum results in single arcs of precipitation in the gamma region.

**Enzyme Activity:** 20 purpurogallin units/ml  
Enzyme activity is determined using 5% pyrogallol (Sigma Product No. P0381) in deionized water, pH 6.0, at 20 °C. One purpurogallin unit will form 1 mg of purpurogallin from pyrogallol in 20 seconds at pH 6.0, 20 °C.

**Titer:** 1:18,000

We are now reporting lot specific information as a titer

by direct ELISA rather than as a working dilution (see below). 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 (Voller, et al.<sup>2</sup>).

Microtiter plates are coated with purified human IgG at a concentration of 5 µg/ml in 0.05M 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. P8287), 0.4mg/ml in 0.05M phosphate-citrate buffer, pH 5.0 containing 0.03% sodium perborate (phosphate-citrate buffer capsules with sodium perborate are available as Sigma Product No. P4922).

#### Working Dilution

Working dilution should be determined by titration assay. Due to differences in assay systems, these titers may not reflect the user's actual working dilution.

#### Storage

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. Wilson, M.B., and P.K. Nakane, Immunofluorescence and Related Staining Techniques (Elsevier/North Holland Biomedical Press, Amsterdam), p 215, (1987).
2. Voller, A., et al., Bulletin WHO, **53**, 55 (1976).

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