

**ANTI-HUMAN IgG (γ-CHAIN SPECIFIC)
PEROXIDASE CONJUGATE
IgG Fraction of Antiserum**Product No. **A8419**

Lot No. 038H4815

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 protein cross linking with 0.2% glutaraldehyde. The conjugate is provided as a solution in 0.01 M phosphate buffered saline, pH 7.4, containing 0.01% thimerosal as a preservative.

Specificity

Specificity for the γ-chain of human IgG is determined by Ouchterlony Double Diffusion (ODD) and immunoelectrophoresis (IEP). The antibody preparation is specific for human IgG when tested against purified human IgA, IgG, 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 the anti-goat IgG and the anti-goat whole serum results in single arcs of precipitation in the gamma region.

Enzyme Activity: 1,000 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 at in 20 seconds at pH 6.0, 20°C.

Molar Ratio (IgG:Peroxidase) = 1.1:1.0**ABPT**

In an agar diffusion assay the conjugate produces a precipitation arc at a dilution of 1:16 versus a 1:160 dilution of human serum.

Titers

1. 1:30,000 (Direct ELISA)

We are now reporting lot specific information as a titer by direct ELISA rather than as a working dilution (see below). Microtiter plates are coated with purified human 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 Sigma Product No. C3041). 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 (Voller, et al.²).

Substrate: o-Phenylenediamine Dihydrochloride (OPD, Sigma Product 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 Sigma Product No. P4922).

2. Dot Blot

- a. A dilution of 1:8,000 was determined in a direct assay using 40 ng human IgG/dot.
- b. A dilution of 1:100,000 was determined in a direct chemiluminescence assay using 10 ng human IgG/dot. Luminol plus enhancer was used as substrate.

3. Immunohistology

A dilution of 1:100 was determined in a direct assay using formalin-fixed, paraffin-embedded human tonsil sections.

Working Dilutions

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

Reference

1. Voller, A., et al., Bulletin WHO, **53**, 55 (1976).

This goat antiserum was maintained at pH 5.0 for 40 minutes to meet U.S.D.A. requirements.

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.

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