

**ANTI-HUMAN IgG (WHOLE MOLECULE)
PEROXIDASE CONJUGATE**
IgG Fraction of AntiserumProduct No. **A8667**

Lot No. 018H4852

Antiserum is developed in goat using human IgG as the immunogen. Whole antiserum is fractionated and then further purified by ion exchange chromatography to provide the IgG fraction of antiserum which is essentially free of other goat serum proteins. Goat anti-human IgG is conjugated to peroxidase by means of a two-step glutaraldehyde method and then purified to remove unconjugated material. The conjugate is supplied as a solution in 0.01 M phosphate buffered saline, pH 7.4, containing 0.01% thimerosal as a preservative.

Specificity

Specificity of the anti-human IgG antiserum is determined by immunoelectrophoresis (IEP) versus human serum and human IgG, prior to conjugation.

Identity and Purity

Identity and purity of the antibody is established by immunoelectrophoresis, 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 in the gamma region.

Molar Ratio (IgG:Peroxidase) = 1.3:1.0**ABPT**

In an agar diffusion assay the conjugate produces a precipitation arc at a dilution of 1:32 versus a 1:320 dilution of normal human serum.

Enzyme Activity: 920 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.

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). 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.)¹. 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).

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 dilution of 1:8,000 was determined by direct dot blot assay using 40 ng human IgG/dot.
- A dilution of 1:80,000 was determined in a direct chemiluminescence assay using 5 ng human IgG/dot. Luminol plus enhancer was used as substrate.

3. Immunohistology

A dilution of 1:200 was determined by direct immunohistology 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.

This goat antisera was maintained at pH 5.0 for 40 minutes to meet USDA requirements.

Reference

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

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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