



Product Information

ANTI-HUMAN IgG (Fc SPECIFIC) PEROXIDASE CONJUGATE Affinity Isolated Antigen Specific Antibody

Product No. **A 0170**

Antiserum is developed in goat using the Fc fragment of human IgG as the immunogen. The antibody is isolated from goat anti-human IgG antiserum by immunospecific purification to remove essentially all goat serum proteins, including immunoglobulins, which do not specifically bind to the Fc fragment of human IgG. Goat anti-human IgG is conjugated to peroxidase by means of a two-step glutaraldehyde method. The product is purified to remove unconjugated material and supplied as a solution in 0.01 M phosphate buffered saline, pH 7.4, containing 0.01% thimerosal as a preservative.

Specificity of the Peroxidase Conjugated Anti-Human IgG is determined by Enzyme Linked Immunosorbent Assay (ELISA). The conjugate is specific for human IgG (Fc fragment) when tested against human IgA, IgG (Fab and Fc fragments), IgM, Bence Jones kappa, and lambda myeloma proteins.

Cross-reactivity of the antibody-conjugate is determined by ELISA. The conjugate shows no reactivity with mouse or rat IgG.

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.

Product Profile

In an agar diffusion assay the conjugate produces a precipitation arc at a dilution of 1:64 versus a 1:640 dilution of normal human serum.

Titers

1. ELISA
A minimum titer of 1:60,000 is determined by direct ELISA. 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.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. 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 Product No. P 4922).

2. Dot Blot
 - a. A minimum dilution of 1:20,000 is determined by direct dot blot assay using 40 ng human IgG/dot.
 - b. A minimum dilution of 1:100,000 is determined in a direct chemiluminescence assay using 20 ng human IgG/dot. Luminol plus enhancer was used as substrate.
3. Immunohistology
A minimum dilution of 1:200 is obtained in a direct assay using formalin-fixed, paraffin-embedded human tonsil sections.

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.

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.

Reference

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

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

PCS 7/00