SIGMA CHEMICAL COMPANY

Product No. A-0170
Lot 114H4804

Anti-Human IgG (Fc Specific) Peroxidase Conjugate

Antibody developed in Goat
Affinity Isolated Antigen Specific Antibody

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

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

Identity and purity of the antibody is estabilised 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.

Molar Ratio (IgG:Peroxidase) = 1.1

ABPT

In an agar diffusion assay the conjugate produces a precipitation arc at a dilution of 1:16 versus a 1:10 dilution of normal human serum.

Dot Blotting: 1:20,000

The working dilution was determined by direct dot blot assay using 40 ng human IgG/dot applied to the nitrocellulose.

Enzyme Activity: 200 purpurogallin units/ml

Enzyme activity is determined using 5% Pyrogallol (Sigma Product No. P-0381) 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: 1:60,000 (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. 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).

Immunohistology: 1:100

The working dilution was obtained 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.

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.

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.