ProductInformation

ANTI-HUMAN IMMUNOGLOBULINS (IgA, IgG and IgM)
Peroxidase Conjugate
Antibody developed in Rabbit
IgG Fraction of Antiserum

Product No. **A8794**

Product Description

Antiserum is developed in rabbit using purified human immunoglobulins as the immunogens. 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 rabbit serum proteins. Rabbit anti-human immunoglobulins is then conjugated to peroxidase by protein cross linking with 0.2% glutaraldehyde.

Specificity for human immunoglobulins is determined by Ouchterlony Double Diffusion (ODD) and immunoelectrophoresis (IEP). The antibody preparation is reactive with human immunoglobulins purified human IgA, IgG, IgM, Bence Jones kappa and Bence Jones lambda myeloma proteins.

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

Reagents

The conjugate is provided as a solution in 0.01 M phosphate buffered saline, pH 7.4, containing 0.01% thimerosal as a preservative.

Product Profile

1. Direct ELISA: Minimum 1:30,000

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.[1]).
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 minimum working dilution of 1:2,000 was determined in a direct assay using 40 ng human IgG, IgA or IgM/dot.
b. A minimum working dilution of 1:20,000 was determined in a direct chemiluminescence assay using 20 ng human IgG, IgA or IgM/dot. Luminol plus enhancer was used as substrate.

3. Immunohistology

A minimum working dilution of 1:200 was determined in a direct assay using formalin-fixed, paraffin-embedded human tonsils.

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.

In an agar diffusion assay the conjugate produces a precipitation arc at a minimum dilution of 1:8 versus a dilution of human serum.

Molar Ratio (IgG:Peroxidase): 0.8-1.5

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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