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Product Information

Anti-Human IgG (Fab specific)−Peroxidase antibody produced in goat
affinity isolated antibody

Catalog Number **A0293**

Product Description

Antiserum is developed in goat using the Fab fragment of human IgG as immunogen. Antibody is isolated from anti-human IgG antiserum by immunospecific purification to remove essentially all goat serum proteins, including immunoglobulins, which do not specifically bind to the Fab fragment of human IgG. Anti-Human IgG is conjugated to peroxidase by means of a two-step glutaraldehyde method and then further purified to remove unconjugated material.

Specificity of the Anti-Human IgG (Fab specific)-Peroxidase is determined by ELISA. Prior to conjugation the antibody is found to react with human serum, human IgA, IgG (whole molecule and Fab fragment), IgM, Bence Jones kappa and lambda myeloma proteins as determined by immunoelectrophoresis (IEP). No reactivity with the Fc fragment of human IgG is observed.

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.

Reagent

Supplied as a solution in 0.01 M phosphate buffered saline pH 7.4, containing 0.05% MIT.

Precautions and Disclaimer

This product is for R&D use only, not for drug, household, or other uses.

Storage

For continuous use, store at 2-8 °C for a maximum of one month. For extended storage, the solution may be frozen in working aliquots at –20 °C. Repeated freezing and thawing, or storage in "frost-free" freezers, is not recommended. If slight turbidity occurs upon prolonged storage, clarify the solution by centrifugation before use.

Product Profile

Molar Ratio (IgG:Peroxidase): 0.6 to 1.5

In an agar diffusion assay the conjugate produces a precipitation arc at a minimum dilution of 1:4 versus a dilution of human serum.

Direct ELISA: Minimum 1:40,000
We are now reporting lot specific information as a titer by direct ELISA rather than as a working dilution. 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 Catalog Number C3041.

Substrate: *o*-Phenylenediamine Dihydrochloride (OPD), Catalog Number P8287), 0.4 mg/ml in 0.05 M phosphate-citrate buffer, pH 5.0 containing 0.03% sodium perborate.
Phosphate-Citrate Buffer with Sodium Perborate capsules are available as Catalog Number P4922.

Dot Blot
A minimum working antibody dilution of 1;100,000 was determined in a direct chemiluminescence assay using 20 ng human IgG/dot. Luminol plus enhancer was used as substrate.

Immunohistochemistry
A minimum working antibody dilution of 1:200 was obtained in a direct assay using formalin-fixed, paraffin-embedded human tonsil sections.

Note: 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).

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