Product No. A-9169
Lot 074H4802

Anti-Rabbit IgG (whole molecule) Peroxidase Conjugate

Antibody Developed in Goat
IgG Fraction of Antiserum

Antiserum is developed in goat using purified rabbit IgG as the immunogen. 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 goat serum proteins. Goat anti-rabbit IgG is then conjugated to peroxidase by protein cross linking with 0.2% glutaraldehyde. The conjugate is provided 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-Rabbit IgG antibodies is determined by immunoelectrophoresis (IEP) versus normal rabbit serum and rabbit IgG.

Identity and Purity

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

Enzyme Activity: 725 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.

ABPT

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

Molar Ratio (IgG:Peroxidase) = 1.2

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]).

1. 1:50,000

Substrate: *o*-Phenylenediamine Dihydrochloride (OPD, Sigma Product No. P-8287), 0.4 mg/ml in 0.05 M phosphate-citrate buffer, pH 5.0 containg 0.03% sodium perborate (Phosphate-Citrate Buffer Capsules with Sodium Perborate are available as Sigma Product No. P-4922).
Microtiter plates are coated with purified rabbit 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).

2. 1:6,000

Substrate: 5-Aminosalicylic Acid (5AS) (Sigma Product No. A-6178).
Microtiter plates are coated with purified rabbit IgG at a concentration of 20 µg/ml in phosphate buffered saline.

Dot Blot

1. A 1:12,000 dilution in a direct assay using 40 ng rabbit IgG/dot.
2. A 1:12,000 dilution in an indirect assay using 20 ng/dot human IgG and rabbit anti-human IgG at 1:1000.

In order to obtain best results, it is recommended that each individual user determine the optimum working dilution for their system by titration assay.

Immunohistology 1:400

The working dilution was determined by indirect immunoperoxidase labeling of formalin-fixed paraffin-embedded human tonsils and rabbit anti-human IgG as the first antibody.

Reference

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

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.

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.