Product No. A-9167
Lot 017H4832

Anti-Guinea Pig IgG (whole molecule)
Peroxidase Conjugate
Antibody developed in Rabbit
IgG Fraction of Antiserum

Antiserum is developed in rabbit using purified guinea pig 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 rabbit serum proteins. Rabbit anti-guinea pig IgG is conjugated to horseradish peroxidase by means of a two-step glutaraldehyde method. The conjugate is provided as a solution in 0.01 M PBS, pH 7.4, containing 0.01% thimerosal as a preservative.

Specificity

Specificity of the Peroxidase Conjugated Anti-Guinea Pig IgG antibodies is determined by immunoelectrophoresis (IEP) versus normal guinea pig serum and guinea pig 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 anti-rabbit IgG and anti-rabbit whole serum results in single arcs of precipitation in the gamma region.

Enzyme Activity: 570 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.

Titers

1. 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]

a. 1:40,000
Microtiter plates are coated with purified guinea pig 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).

b. 1:4,000
Microtiter plates are coated with purified guinea pig IgG at a concentration of 20 µg/ml in phosphate buffered saline.
Substrate: 5-Aminosalicylic Acid (5AS) (Sigma Product No. A-6178).

2. Dot Blot

a. A dilution of 1:10,000 was determined in a direct assay using 40 ng guinea pig IgG/dot.
b. A dilution of 1:4,000 was determined in an indirect assay using 20 ng peroxidase/dot and guinea pig anti-peroxidase the primary antibody.

3. Immunohistology

A dilution of 1:600 was determined by an indirect assay using formalin-fixed, paraffin-embedded human pancreas and guinea pig anti-porcine insulin as the primary antibody.

Working Dilutions

Working dilutions should be determined by titration assay. Due to product improvement and changes in the assay procedure, we now list a lot specific titer by direct ELISA for this product. Due to differences in assay systems, this titer may not reflect the user's actual working dilution.

ABPT

In an agar diffusion assay the conjugate produces a precipitation arc at a dilution of 1:64 versus a 1:160 dilution of guinea pig serum.

Molar Ratio (IgG:Peroxidase) 1.3:1.0

Reference

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

Storage

For continuous use, store at 2-8°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.