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

Product No. **A9292**

Lot No. 018H4827

Antiserum is developed in rabbit using purified horse 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-horse 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-Horse IgG antibodies is determined by immunoelectrophoresis (IEP) versus normal horse serum and horse 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-rabbit IgG and the anti-rabbit whole serum results in single arcs of precipitation in the gamma region.

Enzyme Activity: 1,200 purpurogallin units/ml
Enzyme activity is determined using 5% Pyrogallol (Sigma Product No. P0381) in deionized water, pH 6.0, at 20°C. One purpurogallin unit will form 1 mg of purpurogallin from pyrogallol at in 20 seconds at pH 6.0, 20°C.

Titers
1. Direct ELISA : 1:20,000
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 horse 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).
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 dilution of 1:8,000 was determined in a direct assay using 40 ng horse IgG/dot.
b. A dilution of 1:10,000 was determined in an indirect assay using 20 ng horse IgG/dot and horse anti-human IgG at as the primary antibody.
c. In an indirect chemiluminescence system using 10 ng human IgG/dot and horse anti-human IgG as the primary antibody, this product was determined to have a dilution of 1:100,000 when used as secondary antibody. 1,2-Dioxetane and enhancer was used as substrate.
3. Immunohistology
A dilution of 1:150 was determined by an indirect assay using formalin-fixed, paraffin-embedded human tonsil and horse anti-human IgG as the primary antibody.

ABPT
In an agar diffusion assay the conjugate produces a precipitation arc at a dilution of 1:32 versus a 1:320 dilution of horse serum.

Molar Ratio (IgG: Peroxidase) : 1.2

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.

Reference

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

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. 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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