



Product Information

ANTI-HUMAN IgG (WHOLE MOLECULE) PEROXIDASE CONJUGATE IgG Fraction of Antiserum

Product No. **A 8792**

Product Description

Antiserum is developed in rabbit using purified human 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-human 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 of Peroxidase Conjugated Rabbit Anti-Human IgG is determined by immunoelectrophoresis (IEP) versus normal human serum and human IgG.

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.

Product Profile

Enzyme Activity: At least 450 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 at in 20 seconds at pH 6.0, 20 °C.

Titers

- 1:40,000 to 1:50,000 (Direct ELISA)

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 Product No. C 3041). Titer is defined as the dilu-

tion 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, 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 Product No. P 4922).

2. Dot Blot

- a. A minimum dilution of 1:6,000 was determined in a direct assay using 40 ng human IgG/dot.
- b. A minimum dilution of 1:80,000 was determined in a direct chemiluminescence assay using 10 ng human IgG/dot. Luminol plus enhancer was used as substrate.

3. Immunohistology

A minimum dilution of 1:150 was determined by direct staining of formalin-fixed, paraffin-embedded human tonsil sections.

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.

Molar Ratio (IgG:Peroxidase): 0.8 to 1.5

Reference

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

Storage/Stability

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.

Pcs12/02

Sigma brand products are sold through Sigma-Aldrich, Inc.

Sigma-Aldrich, Inc. warrants that its products conform to the information contained in this and other Sigma-Aldrich publications.

Purchaser must determine the suitability of the product(s) for their particular use. Additional terms and conditions may apply.

Please see reverse side of the invoice or packing slip.