ANTI-DOG IgG (WHOLE MOLECULE)
PEROXIDASE CONJUGATE
ANTIBODY DEVELOPED IN RABBIT
IgG FRACTION OF ANTISERUM

Product No. **A9042**

Lot No. 018H4826

Antiserum is developed in rabbit using purified dog 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-dog 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-Dog IgG antibodies is determined by immunoelectrophoresis (IEP) versus normal dog serum and dog IgG, prior to conjugation.

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.

Molar Ratio (IgG:Peroxidase) = 1.3

Enzyme Activity: 1,380 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 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 1:320 dilution of dog serum.

Titers
1. 1:25,000 (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]). Microtiter plates are coated with purified dog 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 1:3,000 was determined in a direct assay using 40 ng dog IgG/dot.
b. A dilution of 1:40,000 was determined in a direct chemiluminescence assay using 10 ng dog IgG/dot. Luminol plus enhancer was used as substrate.

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

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.

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.

2/98