

**Product No. A-9046**  
**Lot 086H4824**

**Anti-Chicken IgG (whole molecule)**  
**Peroxidase Conjugate**  
Antibody developed in Rabbit  
Affinity Isolated Antigen Specific Antibody

Antiserum is developed in rabbit using purified chicken IgG as the immunogen. Affinity isolated antigen specific antibody is obtained from rabbit anti-chicken antiserum by immunospecific purification which removes essentially all rabbit serum proteins, including immunoglobulins, which do not specifically bind to chicken IgG. Rabbit anti-chicken 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-Chicken IgG antibodies is determined by immunoelectrophoresis (IEP) versus normal chicken serum and chicken 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.

**Enzyme Activity:** 550 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.

**Molar Ratio** (IgG:Peroxidase) = 0.9:1.0

#### **Titers**

1. 1:30,000 (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 chicken 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). 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.<sup>1</sup>).

**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).

2. Dot Blot

- a. A dilution of 1:12,000 was determined in a direct assay using 40 ng chicken IgG/dot.
- b. A dilution of 1:15,000 was determined in an indirect assay using 20 ng human IgG/dot and chicken anti-human IgG as the first antibody.
- c. In an indirect chemiluminescence system using 5 ng human IgG/dot and chicken anti-human IgG as the primary antibody, this product was determined to have a dilution of 1:160,000 when used as secondary antibody. Luminol plus enhancer was used as substrate.

### 3. Immunohistology

A working dilution of 1:1,000 was determined by indirect immunoperoxidase labeling of formalin-fixed, paraffin-embedded human tonsils using chicken anti-human IgG as the first antibody.

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

#### **ABPT**

In an agar diffusion assay the conjugate produces a precipitation arc at a dilution of 1:64 versus a 1:80 dilution of chicken serum.

### **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.

### **Reference**

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