

**Product No. A 5795**  
**Lot 077H4830**

**Anti-Rat IgG (whole molecule)**  
**Peroxidase Conjugate**  
Antibody developed in Rabbit  
Affinity Isolated Antigen Specific Antibody  
Adsorbed with Human IgG

Antiserum is developed in rabbit using purified rat IgG as the immunogen. Antibody is isolated from rabbit anti-rat IgG antiserum by immunospecific purification which removes essentially all rabbit serum proteins, including immunoglobulins, which do not specifically bind to rat IgG. The antibody preparation is solid phase adsorbed with human IgG to ensure minimal cross reactivity in tissue or cell preparations. Rabbit anti-rat IgG is 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 anti-rat IgG is determined by immunoelectrophoresis (IEP) using normal rat serum and rat IgG. The conjugate shows no reaction with human IgG by IEP.

### Identity and Purity

Identity and purity of the antibody is established by immunoelectrophoresis, prior to conjugation. Electrophoresis of the antibody preparation followed by diffusion versus anti-rabbit IgG and anti-rabbit whole serum results in single arcs of precipitation.

**Enzyme Activity:** 230 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.

### ABPT

In an agar diffusion assay the conjugate produces a precipitation arc at a dilution of 1:64 versus a 1:640 dilution of rat serum.

**Molar Ratio** (IgG: Peroxidase) = 1.2

### Titers

1. 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 rat 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 450nm 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 dilution of 1:12,000 was determined in a direct assay using 40 ng rat IgG/dot.
- A dilution of 1:12,000 was determined in an indirect assay using 20 ng human IgG/dot and rat anti-human IgG as the primary antibody.
- In an indirect chemiluminescence system using 10 ng human IgG/dot and rat anti-human IgG as the primary antibody, this product was determined to have a dilution of 1:80,000 when used as secondary antibody. Luminol plus enhancer was used as substrate.

3. Immunohistology

A dilution of 1:750 was determined in an indirect assay using formalin-fixed, paraffin-embedded human tonsil and rat anti-human IgG as the primary antibody.

### **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 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.

### **Reference**

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