

#### Product No. F-9262 Lot 055H4812

# Anti-Rabbit IgG (whole molecule) FITC Conjugate

Antibody Developed in Goat IgG Fraction of Antiserum

Anti-Rabbit IgG is developed in goat using purified rabbit 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 goat serum proteins. Goat anti-rabbit IgG is conjugated to Fluorescein Isothiocyanate (FITC) in an alkaline reaction, then further purified to remove unbound FITC. 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**

The antiserum is determined to be immunospecific for rabbit IgG by immunoelectrophoresis versus normal rabbit serum and rabbit IgG, prior to conjugation.

#### **Identity and Purity**

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

## **Working Dilutions**

- A working dilution of 1:16 was determined by direct immunofluorescent labeling of rabbit spleen cells.
- A working dilution of 1:80 was determined by indirect immunofluorescent labeling of formalinfixed, paraffin-embedded human tonsil sections using rabbit anti-human IgG as the primary antibody.

In order to obtain best results it is recommended that each individual user determine the optimum working dilutions for their system by titration assay. **F/P Molar Ratio:** 3.7

 $A_{280}/A_{495}$ : 1.1

The F/P molar ratio is determined spectrophotometrically as follows:

$$F/P = \underbrace{A_{495} \times 1.4}_{A_{280} - (0.36 \times A_{495}) \times 0.2} \times 0.41$$

Where:

0.2 = The extinction coefficient of bound FITC at a concentration of 1  $\mu g$  per ml at pH 7.2

0.36 = The fluorochrome absorbance correction factor (non-protein absorbance).

#### **ABPT**

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

**Protein Concentration** = 16.1 mg/ml by absorbance at 280nm ( $E_{280}^{1\%} = 14.0$ ).

#### **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.

