

Product No. A-1687

Lot 066H4855

**Monoclonal Anti-Rat κ & λ Light Chains
Peroxidase Conjugate**

Ig Fraction of Mouse Ascites Fluid

Clones RT-39 & RL-6

Peroxidase Monoclonal Anti-Rat κ & λ Light chains is a solution of Monoclonal Anti-Rat κ Light Chain and Monoclonal Anti-Rat λ Light Chain conjugated to horseradish peroxidase. Monoclonal anti-rat κ is derived from the RT-39 hybridoma produced by the fusion of mouse myeloma cells and splenocytes from BALB/c mice immunized with rat kappa light chain. Monoclonal anti-rat λ is derived from the RL-6 hybridoma produced by the fusion of mouse myeloma cells and splenocytes from BALB/c mice immunized with rat lambda light chain. The immunoglobulin fractions of the two ascites fluids are conjugated to peroxidase by protein cross linking with 0.2% glutaraldehyde. The conjugated antibodies are lyophilized from a solution of 1% BSA containing 0.01% thimerosal as preservative.

Specificity

Peroxidase Monoclonal Anti-Rat κ + λ Light Chains reacts specifically with epitopes located on the rat κ light chains and rat λ light chains on the various rat immunoglobulin classes and subclasses. No cross-reaction observed with IgG preparations of human, rabbit, mouse, goat and sheep.

Uses

Peroxidase Monoclonal Anti-Rat κ + λ Light Chains may be used for the localization of light chains on rat immunoglobulins using various immunochemical assays such as ELISA, immunohistology and dot immunobinding assay.

Molar Ratio: (IgG:Peroxidase) 0.9:1.0

Enzyme Activity: 640 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.

Titers

1. 1:40,000 (Direct ELISA)

Microtiter plates are coated with purified rat IgG at a concentration of 5 μ g/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.)¹.

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 1:4,000 was determined in a direct assay using rat IgG or rat κ or λ light chains (50 - 500 ng/dot).
- b. A dilution of 1:20,000 was determined in a direct assay using 50 - 500 ng of rat κ light chains, rat λ light chains or normal rat IgG/dot. Luminol plus enhancer was used as substrate.

3. Immunohistology

A dilution of 1:100 was determined in an indirect assay using formalin-fixed, paraffin-embedded human tonsils. Rat anti-human IgG is used as the primary antibody.

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.

Reconstitution and Storage Instructions

To one vial of lyophilized powder, add 0.5 ml of deionized water. Rotate vial gently until powder dissolves. Prior to reconstitution, store the product at 2-8°C. After reconstitution, the solution may be stored frozen in working aliquots. Repeated freezing and thawing 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).