MONOCLONAL ANTI-PHOSPHOTYROSINE
CLONE PT-66
Biotin Conjugate
Immunoglobulin Fraction of Mouse Ascites Fluid

Product No. **B1531**
Lot 017H4877

Monoclonal Anti-Phosphotyrosine (mouse IgG1 isotype) is derived from the hybridoma produced by the fusion of mouse myeloma cells and splenocytes from an immunized mouse. Phosphotyrosine-BSA was used as the immunogen. The isotype is determined using Sigma ImmunoType™ Kit (Sigma Stock No. ISO-1) and by a double diffusion immunoassay using Mouse Monoclonal Antibody Isotyping Reagents (Sigma Stock No. ISO-2). The immunoglobulin fraction of the ascites fluid is conjugated to ε-amino caproic acid-N-hydroxysuccinimide. This covalent coupling of biotin to the immunoglobulin allows for the binding of Avidin, ExtrAvidin™ or Streptavidin bearing a variety of different labels. The conjugate is provided as a liquid in 0.01 M phosphate buffered saline, pH 7.4, with 1% inactivated BSA and 15 mM sodium azide (see MSDS)* as a preservative.

Specificity
This antibody is specific for phosphorylated tyrosine both as the free amino acid or when conjugated to carriers such as BSA or KLH. It does not react with non-phosphorylated tyrosine or other phosphorylated amino acids, including serine and threonine, nor does it react with phosphorylated molecules such as AMP or ATP.

Protein Concentration: 2.7 mg/ml (prior to addition of BSA).

Uses
The conjugate maybe used as an analytical tool by enabling the identification and quantification of tyrosine-phosphorylated proteins. Since avidin, streptavidin and ExtrAvidin™ show high affinity interaction with biotin, the biotin-avidin system is an extremely effective tool in molecular biology, protein chemistry and immunology. Because of the high specificity of the biotinylated anti body to phosphotyrosine, the stability of the biotin-avidin complex, and the availability of a variety of detection reagents (Avidin, Streptavidin or ExtrAvidin™ conjugated to FITC, TRITC, Peroxidase or Alkaline Phosphatase), the detection and quantitation of tyrosine-phosphorylated proteins can be easily accomplished.

Working Dilutions
1. Direct ELISA: 1:100,000
The working dilution is determined by testing dilutions of the conjugate in microtiter plates coated with Phosphotyrosine-BSA at 10 µg/ml, and using ExtrAvidin™ Peroxidase (Sigma Product No. E-2886) at 2 µg/ml, which gives absorbance value of 1.0 at A_{450} following 30 minutes of substrate conversion.

2. Direct Dot Blot: 1:32,000
The working dilution was determined in a direct assay using 40 ng Phosphotyrosine BSA/dot and ExtrAvidin™ Peroxidase at 1 µg/ml.

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

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.

* Due to the sodium azide content a material safety data sheet (MSDS) for this product has been sent to the attention of the safety officer of your institution. Consult the MSDS for information regarding hazardous and safe handling practices.

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