

Product Specification Sheet

Product: IRDye™ 800 Conjugated Protein A Purified Murine Monoclonal Anti-PhosphoThreonine

Code: 200-332-263

Lot # 13267

Size: 0.1 mg

Clone: 18F6

Isotype: IgG₁ Kappa

Physical State: Lyophilized

Antibody Concentration: 1.0 mg/ml (by UV absorbance at 280 nm)

Label: IRDye™ 800 (MW 1166.2)

Fluorochrome/Protein Ratio: 4.3 moles IRDye™ 800/mole of Mouse IgG

Absorption Wavelength: 774 nm (in PBS)

Emission Wavelength: 800 nm

Buffer: 0.02 M Potassium Phosphate, 0.15 M Sodium Chloride, pH 7.2

Stabilizer: 10 mg/ml BSA IgG and Protease free

Preservative: 0.01% (w/v) Sodium Azide

Application(s): Fluorescence technology is widely used to detect proteins. However, many common visible fluorophores often result in considerable background fluorescence in the visible range. Visible fluorophores are rarely used for membrane-based protein detection because of this high background. IRDye™ 800 antibody and reagent conjugates are specifically designed for protein detection methods that use longer-wavelength, near-infrared (IR) fluorophores to visualize proteins in western blotting and other applications. Very low background fluorescence in the IR range provides for a much higher signal-to-noise ratio than visible fluorophores. Detection levels in the picogram range on Western blots rival the sensitivity of chemiluminescence on film. IRDye™ 800 conjugates are optimized for the Odyssey® Infrared Imaging System developed by LI-COR. IRDye™ 800 conjugates are also suitable for immunofluorescence microscopy using commercially available excitation/emission filters in the 780nm/820nm range. Dual simultaneous labeling in western blots or microscopy is achieved when IRDye™ 800 conjugates are used in conjunction with Cy5.5™ conjugates. IRDye™ 800 conjugates provide an ultra-sensitive and convenient alternative to standard chemiluminescent protein detection methods, as well as a valuable tool for multicolor imaging. This monoclonal antibody reacts specifically with phosphothreonine and shows minimal reactivity by ELISA and competitive ELISA with phosphoserine or phosphotyrosine. The antibody reacts with free phospho amino acid, phosphothreonine conjugated to carriers such as thyroglobulin or BSA, and detects the presence of phosphothreonine in proteins of both unstimulated and stimulated cell lysates. Although not tested, this antibody is likely functional in RIA, flow cytometry, immunohistochemistry and immunoprecipitation. Phosphorylation of threonine residues is associated with many growth factors and oncogene protein kinases, and is important for cell signaling in activation, proliferation and differentiation.

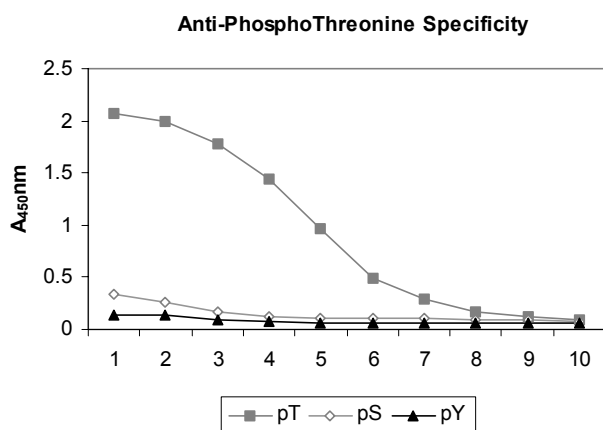


Figure 1. ELISA results of Mab anti-phosphothreonine antibody tested against BSA conjugates of pT, pY and pS. Each well was coated with 0.1 µg of conjugate. The starting dilution of antibody was 1:1000 and each point on the Y-axis represents a 2-fold dilution. HRP conjugated Gt-a-Mouse IgG H&L and TMB substrate were used for detection.

Recommended Dilution(s): This product was tested by immunoblot using a lysate after EGF stimulation of A431 cells separated and transferred to nitrocellulose membrane. A 1:5,000 dilution is sufficient to detect 6-12 pg of threonine phosphorylation of proteins. Researchers should determine optimal titers for other applications.

Storage Conditions: Store vial at 4° C prior to restoration. Restore with 0.1 ml of deionized water (or equivalent). Centrifuge product if not completely clear after standing at room temperature. This product is stable at 4° C as an undiluted liquid. Dilute only prior to immediate use. Expiration date is one (1) year from date of restoration.

Purity and Specificity: This protein A purified mouse monoclonal antibody reacts specifically with phosphothreonine and minimal cross reactivity is observed against phosphoserine or phosphotyrosine.

Immunogen: This monoclonal antibody was produced after repeated immunizations of balb/c mice with phosphothreonine conjugated KLH.

Hybridoma: Produced by the fusion between mouse splenocytes and mouse myeloma SP2/0 cells using conventional hybridoma technology.

Conjugation Reference: LI-COR Biosciences, Lincoln, NE.

General Reference(s):

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Edleman, AM, et al. (1987) Protein serine/threonine kinases. *Annu. Rev. Biochem.* **56**:567.

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Sengupta, A, et al, (1988) Identification and subcellular localization of proteins that are rapidly phosphorylated in tyrosine in response to colony-stimulating factor 1. *Proc. Natl. Acad. Sci. USA* **85**:8062.

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Jin, DY, et al, (1998) Human T cell leukemia virus type 1 oncoprotein Tax targets the human mitotic checkpoint protein MAD1. *Cell.* **93**:81-91.

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Fleming, I.N., et al, (1999) Ca²⁺/calmodulin-dependent protein kinase II regulates Tiam1 by reversible protein phosphorylation. *J. Biol. Chem.* **274**(18):12753-12758.

Related Product(s):

#500-301-263	Murine Mab anti-PhosphoThreonine Ascites
#500-301-262	Murine Mab anti-PhosphoTyrosine Ascites
#200-332-262	IRDye800 Conjugated Protein A Purified Monoclonal anti-Phospho-Tyrosine (Mouse)
#200-332-263	IRDye800 Conjugated Protein A Purified Monoclonal anti-Phospho-Threonine (Mouse)
#200-332-246	IRDye800 Conjugated Protein A Purified Murine Monoclonal Anti-Rhodamine
#610-132-121	IRDye800 Conjugated Affinity Purified Anti-MOUSE IgG (H&L) (GOAT) (MX10)

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