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Certificate of Analysis

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

Code: 200-332-262

Lot # 21076

Size: 0.1 mg

Clone: 13F9

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: 2.3 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 phosphotyrosine and shows minimal reactivity by ELISA and competitive ELISA with phosphoserine or phosphothreonine. The antibody reacts with free phospho amino acid; phosphotyrosine conjugated to carriers such as thyroglobulin or BSA, and detects the presence of phosphotyrosine 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 tyrosine residues is associated with many growth factors and oncogene protein kinases, and is important for cell signaling in activation, proliferation and differentiation.

Recommended Dilution(s):

LI-COR Odyssey® BLOT

1:5,000 - 1:25,000

LI-COR In-Cell Western®

User Optimized

OTHER APPLICATIONS

User Optimized

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 phosphotyrosine and minimal cross reactivity is observed against phosphoserine or phosphothreonine.

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

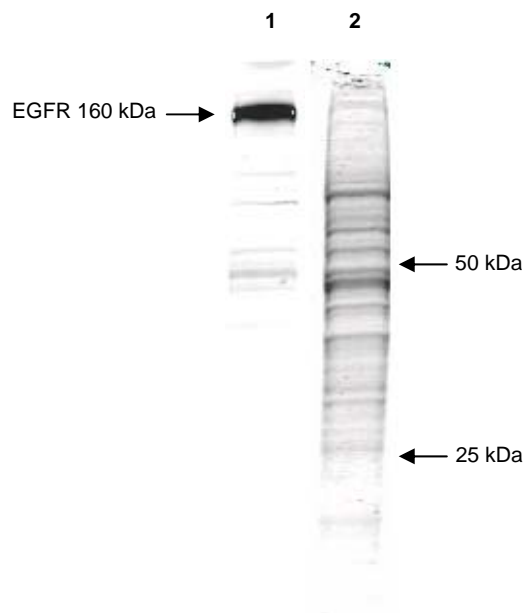


Figure 1. Mab anti-phosphotyrosine antibody (clone 13F9) is shown to detect by immunoblot (lane 1) phosphorylated EGFR in an A431 cell lysate (10 μ g per lane) after stimulation with EGF. The binding of EGF to EGFR causes rapid activation of its intrinsic autophosphorylation of multiple tyrosine residues in the cytoplasmic domain of the protein. This propagates the ERK1/2 signal pathway which regulates cell growth, survival, proliferation and differentiation. Lane 2 shows total protein in the lysate after coomassie staining. Lane 3 (not shown) shows minimal reactivity of the antibody with a lysate from an unstimulated A431 cell lysate. A 4-20% gradient gel was used for separation prior to transfer to nitrocellulose. A 1:1,000 dilution of Mab anti-phosphotyrosine is used at room temperature for 60 min followed by detection using IRDye™ 800 Conjugated Goat-a-Mouse IgG [H&L] (610-132-120) diluted 1:2,500 for 30' at room temperature. Image was processed and captured using the Odyssey® Infrared Imaging System developed by LI-COR. IRDye is a trademark of LI-COR, Inc. Other detection systems will yield similar results.

General Reference(s):

Sarmay, G., et al., (1994) Protein-tyrosine kinase activity tightly associated with human type II Fc gamma receptors. *Proc. Natl. Acad. Sci. USA* **91**, 4140.

Arad-Dann, H., (1993) Immunohistochemistry of phosphotyrosine residues: identification of distinct intracellular patterns in epithelial and steroidogenic tissues. *J. Histochem. Cytochem.* **41**, 513.

Dvir, A., et al., (1991) The inhibition of EGF-dependent proliferation of keratinocytes by tyrphostin tyrosine kinase blockers. *J. Cell Biol.* **113**, 857.

Far, D.F., et al., (1994) Immunofluorescent quantification of tyrosine phosphorylation of cellular proteins in whole cells by flow cytometry. *Cytometry* **15**, 327.

Barry, S.T., and Critchley, D.R. (1994) The RhoA-dependent assembly of focal adhesions in Swiss 3T3 cells is associated with increased tyrosine phosphorylation and the recruitment of both pp125FAK and protein kinase C-delta to focal adhesions. *J. Cell Sci.* **107**, 2033.

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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