

Anti-Phosphotyrosine

Polyclonal Antibody

Cat. # 06-427

Lot # 32815

pack size: 200 µg

Store at -20°C

FOR RESEARCH USE ONLY



Certificate of Analysis

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Applications	Species Cross-Reactivity	Antibody Isotype	Epitope/Region	Host Species	Molecular Weight	Accession #
WB, IP	H, M, R	IgG	N/A	Rb	Varies	N/A

Background

The phosphorylation of specific tyrosine residues has been shown to be a primary mechanism of signal transduction during normal mitogenesis, cell cycle progression and oncogenic transformation, its role in other areas such as differentiation and gap junction communication, is a matter of active and ongoing research. Antibodies that specifically recognize phosphorylated tyrosine residues have proved to be invaluable to the study of tyrosine phosphorylated proteins and the biochemical pathways in which they function.

Presentation

Purified rabbit polyclonal IgG in storage buffer containing 0.2 M Tris-glycine, Ph 7.2, 0.15 M NaCl, 1 mg/mL of BSA containing 0.05% sodium azide. Frozen at -20°C.

Concentration

181.81 mg/mL

Specificity

The immunoreactivity of the antibody is totally inhibited by the use of 100 mM phenyl phosphate, a phosphotyrosine analog. The phosphotyrosine antibody is purified by immunoaffinity chromatography using either a dual phospho-peptide gel or a BSA-phosphotyrosine gel. All of the phosphotyrosine immunoreactivity present in the antisera is immunoabsorbed whether the antibody is purified by either gel indicating that the antibody is not sequence-specific but specific for phosphotyrosine residues

Species Cross-reactivity

Human, mouse and rat. Other species cross-reactivity is unknown.

Immunogen

In order to produce broad spectrum polyclonal phosphotyrosine antibodies, rabbits were

immunized with three phosphorylated immunogens: (1) phosphotyrosine covalently linked to KLH; (2) the c-Src carboxyl terminal regulatory phosphopeptide (TS-T-E-P-Q-pY-Q-P-G-E-N-L; Catalog # 12-218) covalently linked to KLH, and; (3) a phosphopeptide associated with high tyrosine kinase activity in human lymphocytes (R-R-L-I-E-D-A-E-pY-A-A-R-G; Catalog # 12-217) covalently linked to KLH. Both of the phosphopeptide haptens serve as strong substrates for tyrosine phosphatases and are part of the two colorimetric tyrosine phosphatase kits provided by Upstate, Inc. (Catalog # 17-125, 17-126).

Molecular Weight

Varies

Method of Purification

Protein A Purified followed by immunoaffinity

Storage and Handling

Stable for 1 year at -20°C from date of receipt.

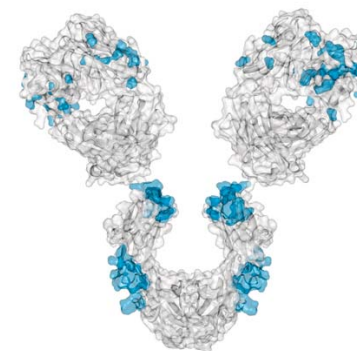
Handling Recommendations: Upon receipt, and prior to removing the cap, centrifuge the vial and gently mix the solution. Aliquot into microcentrifuge tubes and store at -20°C. Avoid repeated freeze/thaw cycles, which may damage IgG and affect product performance.

Control

Included Positive Antigen Control: Catalog # 12-302, EGF-stimulated A431 cell lysate. Add 2.5 µL of 2-mercaptoethanol/100 µL of lysate and boil for 5 minutes to reduce the preparation. Load 20 µg of reduced lysate per lane for minigels.

Quality Control Testing

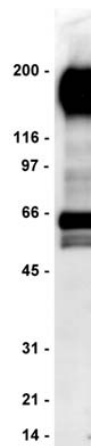
Evaluated by western blot on EGF-stimulated human A431 carcinoma cells.



References

1. Payne, E., *et al.* (2001). *J Virol.* 75:4150-7.
2. Yamagishi, S. I., *et al.* (2001). *Diabetes.* 50:1491-4.
3. Luttrell, D.K., *et al.* (1994). *Proc. Natl. Acad. Sci. USA.* 91: 83.
4. Song, Z., *et al.* (1993). *Cell.* 72:767.
5. Trevillyan, J.M., *et al.* (1985). *Biochim. Biophys. Acta.* 845: 1.

Western Blot Analysis: 0.5-2 µg/mL of this antibody detected proteins containing phosphotyrosine residues in RIPA lysates from EGF-stimulated human A431 carcinoma cells.



Western Blot Analysis:
Representative lot data. EGF-stimulated A431 cell lysate was resolved by electrophoresis, transferred to nitrocellulose and probed with anti-Phosphotyrosine (0.5 µg/mL). Proteins were visualized using a goat anti-rabbit secondary antibody conjugated to HRP and a chemiluminescence detection system.

APPLICATION LEGEND: WB Western Blotting IP Immunoprecipitation IC Immunocytochemistry IF Immunofluorescence
IH Immunohistochemistry (Tissue)

SPECIES LEGEND: H Human M Mouse R Rat Rb Rabbit WR Most Common Vertebrates

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Additional Research Applications

Immunoprecipitation: 4 µg of a previous lot immunoprecipitated proteins containing phosphotyrosine residues from a human A431 RIPA lysate.

PROTOCOL**Western Blot**

1. Perform SDS-polyacrylamide gel electrophoresis (SDS-PAGE) on a cell lysate sample (cell lysis buffer: 50 mM Tris-HCl, pH 7.4; 1% NP-40; 0.25% sodium deoxycholate; 150 mM NaCl; 1 mM EDTA; 1 mM PMSF; 1 µg/mL aprotinin, leupeptin, pepstatin; 1 mM Na₃VO₄; 1 mM NaF) and transfer the proteins to nitrocellulose. Wash the blotted nitrocellulose twice with water.
2. Block the blotted nitrocellulose in freshly prepared PBS containing 3% nonfat dry milk (Catalog # 20-200) and 0.05% Tween-20 (PBST-MLK) for 20 minutes at room temperature with constant agitation.
3. Incubate the nitrocellulose with 0.5-2 µg/mL of Anti-Phosphotyrosine, diluted in freshly prepared PBST-MLK, overnight with agitation at 4°C.
4. Wash the nitrocellulose twice with water.
5. Incubate the nitrocellulose in the secondary reagent of choice (a goat anti-rabbit HRP conjugated IgG, Catalog # 12-348, 1:5000 dilution, was used) in PBST-MLK for 1.5 hours at room temperature with agitation.
6. Wash the nitrocellulose with water twice.
7. Wash the nitrocellulose in PBS-0.05% Tween 20 for 3-5 minutes.
8. Rinse the nitrocellulose in 4-5 changes of water.
9. Use detection method of choice (enhanced chemiluminescence was used).

Immunoprecipitation

1. Add 4 µg of anti-Phosphotyrosine and 60 µL (30 µL packed beads) of washed Protein A agarose bead slurry (Catalog # 16-125) to 500 µL of PBS in a microcentrifuge tube.
2. Gently rock the reaction mixture at 4°C for 1 hour.
3. Collect the agarose beads by pulsing (5 seconds in the microcentrifuge at 14,000 x g), and drain off the supernatant. Wash the beads 3 times with either ice-cold cell lysis buffer or PBS.
4. Dilute the cell lysate to roughly 1 µg/µL total cell protein with PBS.
5. Add 500 µg-1 mg cell lysate to the reaction mixture.
6. Gently rock the reaction mixture at 4°C for 1 hour.
7. Collect the agarose beads by pulsing (5 seconds in the microcentrifuge at 14,000 x g), and drain off the supernatant. Wash the beads 3 times with either ice-cold cell lysis buffer or PBS.
8. Resuspend the agarose beads in 60 µL 2X Laemmli sample buffer.
9. Store the beads frozen for future analysis or boil the beads for 5 minutes.
10. Collect the beads after boiling using a microcentrifuge pulse.
11. Perform SDS-PAGE and western blot analysis on a sample of the supernatant fraction.

RELATED PRODUCTS (specific)

cat #	description
AB1599	■ Anti-Phosphotyrosine
MAB3109	■ Anti-Phosphotyrosine, clone 3-365-10
MAB3080B	■ Anti-Phosphotyrosine, Clone PY20, Biotin Conjugated
MAB3080P	■ Anti-Phosphotyrosine, Clone PY20, HRP Conjugated
12-440	■ Poly (Glu4-Tyr) Peptide, biotin conjugate
SGT200	■ Protein Tyrosine Kinase Peptide Substrate
12-217	■ Tyrosine Phosphopeptide (RRLIEDAePYAARG]
12-218	■ Tyrosine Phosphopeptide (TSTEPQpYQPGENL)
APT184	■ Omni-Phos®
SGT410	■ Tyrosine Kinase Activity Assay
12-110	■ Phosphotyrosine control (EGF-stim A431 cell lysate)
12-256	■ Phosphotyrosine Molecular Weight Standards
12-348	■ Goat Anti-Rabbit IgG, HRP conjugate

RELATED PRODUCTS (non-specific)

cat #	description
IPVH00010	■ Immobilon-P 26.5 cm x 3.75 m Roll PVDF 0.45 µm
IPFL00010	■ Immobilon-FL 26.5 cm x 3.75 m Roll PVDF 0.45 µm
IPVH07850	■ Immobilon-P 7 x 8.4 cm PVDF 0.45 mm (sheet) 50/pk
ISEQ00010	■ Immobilon-P SQ 26.5 cm x 3.75 m 1 roll PVDF 0.2 µm
ISEQ07850	■ Immobilon-P 7 x 8.4 cm PVDF 0.2 mm (sheet) 50/pk
IPFL07810	■ Immobilon-FL 7 x 8.4 cm PVDF 0.45 mm (sheet) 10/pk
WBKLS0100	■ Immobilon Western Chemilum HRP Substrate 100 mL
17-373	■ Spray & Glow™ ECL WB Detection System 1 ea
2060	■ Re-Blot Western Blot Recycling Kit
2500	■ Re-Blot Plus Western Blot Recycling Kit
B2080-175GM	■ Blot Quick Blocker Membrane Blocking Agent 175G

■ antibodies ■ Multiplex products ■ biotools ■ cell culture ■ enzymes ■ kits ■ proteins/peptides ■ siRNA/cDNA products

Please visit www.millipore.com for additional product information, test data and references

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