

Anti-Phosphotyrosine, clone 4G10[™]

Monoclonal Antibody

Cat. # 05-321

Lot # DAM1423768

pack size: 100 µg

Store at 2-8°C

FOR RESEARCH USE ONLY
NOT FOR USE IN HUMANS

DO NOT FREEZE



Certificate of Analysis

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Applications	Species Cross-Reactivity	Antibody Isotype	Epitope/Region	Host Species	Molecular Weight	Accession #
WB, IP	A	IgG2bk	N/A	M	Varies	N/A

Background

Some of the tyrosine residues can be tagged with a phosphate group (phosphorylated) by protein kinases. (In its phosphorylated state, it is referred to as phosphotyrosine.). Tyrosine phosphorylation is considered as one of the key steps in signal transduction and regulation of enzymatic activity.

The advent of anti-phosphotyrosine antibodies is one of significant events in signal transduction research. Before the availability of anti-phosphotyrosine antibodies, tyrosyl phosphorylation of proteins and enzymes was investigated through hazardous and time-consuming radioactive experiments. Anti-phosphotyrosine antibodies are commonly used in western blots after the targeted proteins have been immunoprecipitated to measure the tyrosyl phosphorylation of the proteins. Anti-phosphotyrosine antibodies are also directly used on cell lysate to examine the overall change of tyrosine phosphorylation level in response to various treatments.

Presentation

Purified mouse monoclonal IgG2bk in buffer containing 0.02 M phosphate buffer, pH 7.6, 0.25 M NaCl, 0.1% sodium azide. Liquid at 2-8°C.

IgG_{2bk} mouse monoclonal antibody produced *in vitro* by mouse-mouse hybridoma 4G10[®] (FOX-NY [NS-1 derivative] myeloma x spleen cells).

Concentration

1 mg/mL

Specificity

Recognizes tyrosine-phosphorylated proteins from all species.

Species Cross-reactivity

All species

Immunogen

Phosphotyramine-KLH

Molecular Weight

Dependent upon the molecular weight of the tyrosine phosphorylated protein being detected.

Method of Purification

Protein G-Sepharose chromatography

Storage and Handling

Stable for 1 year at 2-8°C from date of receipt.

NOTE: DO NOT FREEZE. For maximum recovery of the product, centrifuge the original vial prior to removing the cap. If the product has accidentally been frozen and thawed, spin it at 13,000 x g for 10 minutes at 2-8°C. Save the supernatant for application.

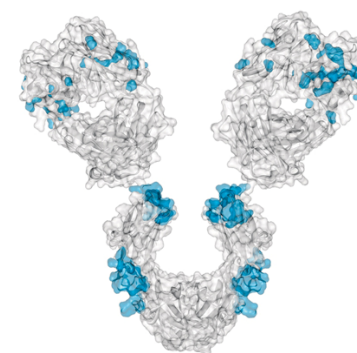
Control

Untreated A-431 (negative control) and EGF treated A-431 (positive control) whole cell lysates.

Included Positive Antigen Control: Catalog # 12-302, EGF-stimulated A431 cell lysate is provided as a free positive antigen control for western immunoblotting. Aliquot as desired, refreeze immediately, and store at -20°C. The lysate is stable for 6 months at -20°C. Before use, 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 immunoblot analysis.

Quality Control Testing

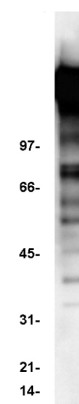
Routinely evaluated on EGF-treated human A431 carcinoma cells.



References

1. Kanakura, Y., *et al.* (1991). *J. Biol. Chem.* 266: 490.
2. Cohen, B., *et al.* (1990). *Proc. Natl. Acad. Sci. USA.* 87: 4458.
3. Druker, B. J., *et al.* (1989). *New Eng. J. Med.* 321: 1383.

Western Blot Analysis: 0.5-2 µg/mL of this lot detected tyrosine-phosphorylated proteins in a modified RIPA lysate from EGF-treated human A431 carcinoma cells (Cohen, B., 1990; , Druker, B. J., 1989; Kanakura, Y., 1991).



Western Blot Analysis:

EGF-stimulated A431 cell lysate was resolved by electrophoresis, transferred to nitrocellulose and probed with anti-phosphotyrosine (1 µg/mL).

Proteins were visualized using a goat anti-mouse 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 A All Species

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Additional Research Applications

Immunoprecipitation: 2-4 µg of a previous lot immunoprecipitated quantitatively the phosphotyrosine containing proteins in the lysate of a confluent culture (10 cm dish) of cells expressing an activated tyrosine kinase. To preserve phosphotyrosine, add: 0.2 mM sodium orthovanadate to the lysis buffer.

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 TBS containing 3% nonfat dry milk (Catalog # 20-200), (TBS-MLK) for 45-90 minutes at room temperature with constant agitation.
3. Incubate the nitrocellulose with 0.5-2 µg/mL of anti-Phosphotyrosine, clone 4G10®, diluted in freshly prepared TBS-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-mouse HRP conjugated, Catalog # 12-349, 1:5000 dilution, was used) in TBS-MLK for 1.5 hours at room temperature with agitation.
6. Wash the nitrocellulose with water twice.
7. Wash the nitrocellulose in TBS-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 with a 30 second exposure was used).

Immunoprecipitation

1. Add 2-4 µg of anti-Phosphotyrosine, clone 4G10® and 60 µL (30 µL packed beads) of washed Protein G agarose bead slurry (Catalog # 16-266) to 500 µL of TBS in a microcentrifuge tube.
2. Gently rock the reaction mixture at 4°C for 1 ho ur.
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 TBS.
4. Dilute the cell lysate to roughly 1 µg/µL total cell protein with TBS.
5. Add 500 µg-1 mg cell lysate to the reaction mixture.
6. Gently rock the reaction mixture at 4°C for 1 ho ur.
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 TBS.
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 immunoblot analysis on a sample of the supernatant fraction

RELATED PRODUCTS (specific)

cat #	description
16-104	■ Anti-Phosphotyrosine, clone 4G10®, FITC conjugate
16-199	■ Anti-Phosphotyrosine, recombinant clone 4G10®, agarose conjugate
12-110	■ Phosphotyrosine control (EGF-stim A431 cell lysate)
16-105	■ Anti-Phosphotyrosine, clone 4G10®, HRP conjugat
12-349	■ Goat Anti-Mouse IgG, HRP conjugate
05-321	■ Anti-Phosphotyrosine, clone 4G10®
05-321X	■ Anti-Phosphotyrosine, clone 4G10®
16-204	■ Anti-Phosphotyrosine, recombinant clone 4G10®, biotin conjugate
16-101	■ Anti-Phosphotyrosine, clone 4G10®, agarose conjugate
16-184	■ Anti-Phosphotyrosine, recombinant clone 4G10®, HRP conjugate
17-153	■ Anti-Phosphotyrosine Immunoblotting Kit (4G10®), ECL Detection
05-777	■ Anti-Phosphotyrosine, recombinant clone 4G10®
17-123	■ Anti-Phosphotyrosine Immunoblotting Kit (4G10®), HRP conjugate)
06-427	■ Anti-Phosphotyrosine
16-103	■ Anti-Phosphotyrosine, clone 4G10®, biotin conjugate
12-256	■ Phosphotyrosine Molecular Weight Standards

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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