

4G10[®] Platinum, Anti-Phosphotyrosine (mouse monoclonal cocktail IgG2b)

Monoclonal Antibody

Cat. # 05-1050

Lot # DAM1581058

pack size: 100 µL

Store at 2-8°C
DO NOT FREEZE

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, IF, IH	A	IgG2b	N/A	M	Varies	N/A

Background

The development of the anti-phosphotyrosine, clone 4G10[®] in 1989 was a monumental discovery for researchers. 4G10[®] was the first and is the best single monoclonal antibody for the detection tyrosine phosphorylation. 4G10[®] is well known for its sensitivity and its ability to detect multiple tyrosine phosphorylations on numerous substrates. It has been validated by thousands of scientific and medical researchers in virtually every application and tyrosine target over the past 2 decades. To improve on something that hundred have tried and no one has succeeded, we pooled 4G10[®] with the next most highly regarded antiphosphotyrosine, clone PY20 to make 4G10[®] Platinum. PY20 itself is a very poor substitute for 4G10[®], but its additive effect allow for a greater level of detection on more substrates that even 4G10[®] alone was not capable of.

Presentation

Proprietary mixture of protein G purified mouse monoclonals 4G10[®] (IgG2b κ) and PY20 (IgG2b) in 0.1 M Tris-glycine, Ph 7.4, 0.15 M NaCl, 0.05% sodium azide.

Liquid at 2-8°C.

Specificity

Tyrosine-phosphorylated proteins from all species

Immunogen

4G10[®] used Phosphotyramine coupled to KLH and PY20 used phosphotyrosine conjugated to carrier protein

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. 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.

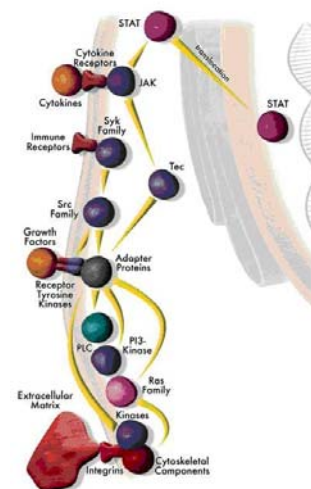
Control

Suggested Positive Antigen Control: Cat. # 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 per 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

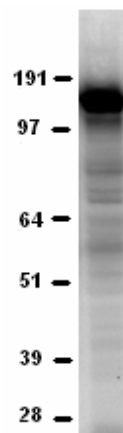
Evaluated by Western Blot on a modified RIPA lysate from EGF-treated human A431 carcinoma cells.

Western Blot Analysis: 1:1,000-1:2,000 of this antibody detected tyrosinephosphorylated proteins in a modified RIPA lysate from EGF-treated human A431 carcinoma cells.



References

1. Foubert, Philippe, *et al.* (2007). *J. Clin. Invest.* 117(6): 1527-1537.
2. Lo, Huey-Ming, *et al.* (2007). *Biochem pharmacol.* 74: 54-63.
3. Nishida, K., *et al.* (2005). *J Cell Biol.* 170: 115-26.
4. Richard, S., *et al.* (2005). *Biochem J.* 388: 379-86.
5. Meyer, R. D., *et al.* (2004). *J Biol Chem.* 279: 735-42.



Western Blot Analysis:
Representative lot data. EGF-stimulated A431 cell lysate (Cat. No.12-302) was resolved by SDS-PAGE and transferred to PVDF membrane. The blot was probed with 4G10[®] Platinum, antiphosphotyrosine (1:1,000). 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: A All Species H Human M Mouse R Rat Rb Rabbit WR Most Common Vertebrates

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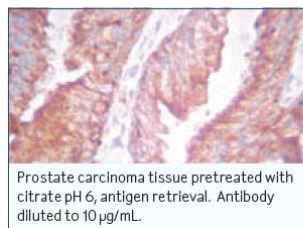
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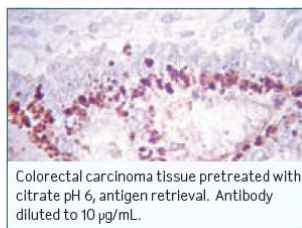
Additional Research Applications

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

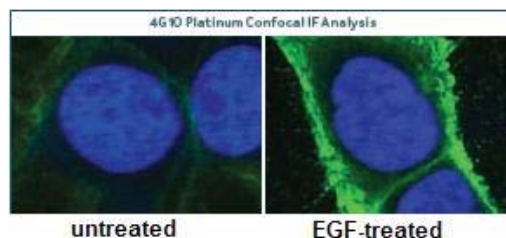
4G10 Platinum IHC Staining



Prostate carcinoma tissue pretreated with citrate pH 6, antigen retrieval. Antibody diluted to 10 µg/mL.



Colorectal carcinoma tissue pretreated with citrate pH 6, antigen retrieval. Antibody diluted to 10 µg/mL.



untreated

EGF-treated

Immunohistochemistry: Tissue samples were pre-treated with citrate, pH 6, antigen retrieval. IHC stained with 10 mg/mL of anti-phosphotyrosine 4G10® Platinum.

Immunofluorescence: A431 cells either untreated (left) or EGF-treated (right) and stained with 4G10® Platinum (green) and DAPI (nuclei, Blue). Cells were visualized on confocal immunofluorescent microscope.

PROTOCOL

Western Blot

1. Perform SDS-PAGE on a cell lysate sample and transfer the proteins to PVDF. Wash the blotted PVDF twice with TBST.
2. Block the blotted PVDF 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 PVDF with 1:1,000 dilution of 4G10® Platinum, anti-Phosphotyrosine diluted in freshly prepared TBST/Milk with agitation for 1 hour at room temperature or overnight at 4°C.
4. Wash the PVDF 4 times with TBST.
5. Incubate the blotted PVDF in the secondary reagent of choice (a goat anti-mouse HRP conjugated, Catalog # 12-349, 1:4000 dilution, was used) in TBS/Milk for 1 hour at room temperature with agitation.
6. Wash the PVDF in TBS-0.05% Tween®-20 for 3-5 minutes.
7. Use detection method of choice (enhanced chemiluminescence with a 30 second exposure was used).

Immunoprecipitation

1. Add 5 µL of 4G10® Platinum, anti-Phosphotyrosine and 60 µL (30 µL packed beads) of washed Protein G agarose bead slurry (Catalog # 16-266) to 500 µL of PBS 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 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 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 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 immunoblot analysis on a sample of the supernatant fraction.

RELATED PRODUCTS (specific)

cat #	description
05-1050ML	4G10® Platinum
05-321	Anti-Phosphotyrosine, clone 4G10®
05-321MG	Anti-Phosphotyrosine, clone 4G10®
05-1000	Anti-Phosphoserine, clone 4A4
05-1000MG	Anti-Phosphoserine, clone 4A4
05-1000X	Anti-Phosphoserine, clone 4A4
17-499	4G10® Platinum/4A4 Phosphopack
16-105	Anti-Phosphotyrosine, clone 4G10®, HRP conjugate
16-104	Anti-Phosphotyrosine, clone 4G10®, FITC conjugate
16-101	Anti-Phosphotyrosine, clone 4G10®, agarose conjugate
16-103	Anti-Phosphotyrosine, clone 4G10®, biotin conjugate
05-947	Anti-Phosphotyrosine, clone PY20

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 Chemillum 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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