

# 4G10<sup>®</sup> Platinum, Anti-Phosphotyrosine

(mouse monoclonal cocktail IgG<sub>2b</sub>)

Monoclonal Antibody

**Cat. # 05-1050**

Lot # R0709B0207

**pack size: 100 µL**

FOR RESEARCH USE ONLY  
NOT FOR USE IN HUMANS

Store at 2-8°C  
**DO NOT FREEZE**



Applications	Species Cross-Reactivity	Antibody Isotype	Epitope/Region	Host Species	Molecular Weight	Genebank Accession#
WB, IP, IC, FC, IF, IH ELISA, FP, IPK	A	IgG2b	N/A	M	Varies	N/A

## Background

The development of the anti-phosphotyrosine, clone 4G10<sup>®</sup> in 1989 was a monumental discovery for researchers. 4G10<sup>®</sup> was the first and is the best single monoclonal antibody for the detection tyrosine phosphorylation. 4G10<sup>®</sup> 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<sup>®</sup> with the next most highly regarded anti-phosphotyrosine, clone PY20 to make 4G10<sup>®</sup> Platinum. PY20 itself is a very poor substitute for 4G10<sup>®</sup>, but its additive effect allow for a greater level of detection on more substrates that even 4G10<sup>®</sup> alone was not capable of.

## Presentation

100 µL of a proprietary mixture of protein G purified mouse monoclonals 4G10<sup>®</sup> (IgG2bk) 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<sup>®</sup> used Phosphotyramine coupled to KLH and PY20 used phosphotyrosine conjugated to carrier protein.

## Method of Purification

Protein G-Sepharose chromatography

## Molecular Weight

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

## Storage/Handling

2 years at 2-8°C from date of shipment.

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

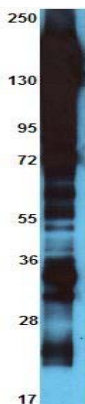
Included Positive Antigen Control: Cat.# 12-302, EGF-stimulated A431 cell lysate is provided as a free positive antigen control for western immunoblotting. Aliquot as desired, refreeze immediately, and store at -20°C. The lysate is stable for 6 months at -20°C. Before use, add 2.5 mL of 2-mercaptoethanol per 100 mL of lysate and boil for 5 minutes to reduce the preparation. Load 20 mg of reduced lysate per lane for immunoblot analysis.

## Quality Control Testing

Routinely evaluated by immunoblot on a modified RIPA lysate from EGF-treated human A431 carcinoma cells

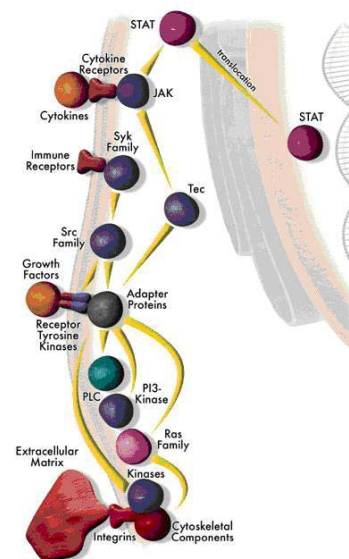
## Immunoblot Analysis:

1:1,000-1:2,000 of this lot detected tyrosine-phosphorylated proteins in a modified RIPA lysate from EGF-treated human A431 carcinoma cells.



## Immunoblot Analysis:

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<sup>®</sup> Platinum, anti-phosphotyrosine (1:1,000). Proteins were visualized using a goat anti-mouse secondary antibody conjugated to HRP and a chemiluminescence detection system.



## 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.

## Immunoprecipitation:

2-4 µL of this 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.

## Additional Referenced Research Applications

Immunofluorescence, Immunohistochemistry (See data on pg.2)

**TESTED APPLICATION LEGEND:** WB Western Blotting IP Immunoprecipitation IC Immunocytochemistry IF Immunofluorescence IH Immunohistochemistry (Tissue) FC Flow Cytometry ELISA Enzyme-linked Immunosorbent Assay FP Fluorescence Polarization IPK IP-Kinase Assay

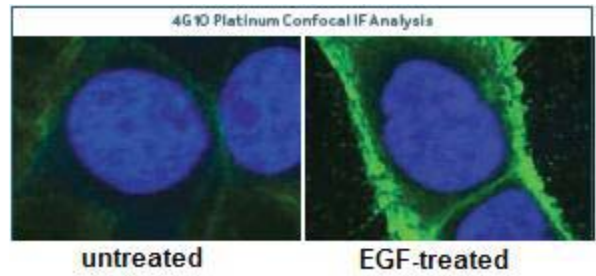
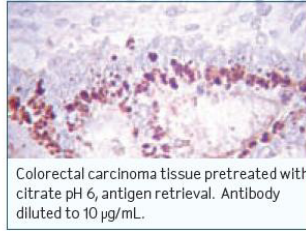
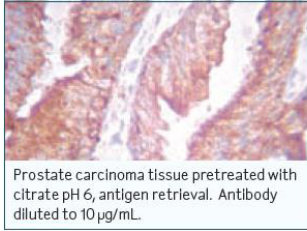
**SPECIES LEGEND:** H Human M Mouse R Rat Rb Rabbit WR Most Common Vertebrates A All Species

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**4G10 Platinum IHC Staining**



**Immunohistochemistry:** Tissue samples were pre-treated with citrate, pH 6, antigen retrieval. IH stained with 10 µg/mL of anti-phosphotyrosine 4G10<sup>®</sup> Platinum.

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

**PROTOCOL**

**Immunoblot**

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<sup>®</sup> 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<sup>®</sup>-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<sup>®</sup> 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 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 immunoblot analysis on a sample of the supernatant fraction.

**RELATED PRODUCTS (specific)**

cat #	description
05-1050X	4G10 <sup>®</sup> Platinum
05-1050ML	4G10 <sup>®</sup> Platinum
05-321	Anti-phosphotyrosine, clone 4G10
05-321MG	Anti-Phosphotyrosine, clone 4G10 <sup>®</sup>
05-1000	Anti-Phosphoserine, clone 4A4
05-1000MG	Anti-Phosphoserine, clone 4A4
17-499	4G10 <sup>®</sup> Platinum/4A4 Phosphopack
16-105	Anti-Phosphotyrosine, clone 4G10 <sup>®</sup> , HRP conjugate
16-104	Anti-Phosphotyrosine, clone 4G10 <sup>®</sup> , FITC conjugate
16-101	Anti-Phosphotyrosine, clone 4G10 <sup>®</sup> , agarose conjugate
16-103	Anti-Phosphotyrosine, clone 4G10 <sup>®</sup> , biotin conjugate
05-947	Anti-Phosphotyrosine, clone PY20
17-315	Tyrosine Kinase Assay Kit, Colorimetric Detection
17-331	Tyrosine Kinase Assay Kit, Chemiluminescence Detection
17-153	Anti-Phosphotyrosine Immunoblotting Kit (4G10 <sup>®</sup> ), ECL Detection
17-123	Anti-Phosphotyrosine Immunoblotting Kit (4G10 <sup>®</sup> ), HRP conjugate
05-1000X	Anti-Phosphoserine, clone 4A4

**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 <sup>™</sup> 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
12-302	EGF-Stimulated A431 Cell Lysate
12-349	Goat Anti-Mouse IgG, HRP conjugate
12-110	Phosphotyrosine control (EGF-stim A431 cell lysate)

Antibodies Beadlyte<sup>®</sup> products biotools cell culture enzymes kits proteins/peptides siRNA/cDNA products

Please visit [www.millipore.com](http://www.millipore.com) for additional product information, test data and references

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4G10 material is covered under US patent number 6,824,989 and related foreign patents.