

Anti-Phosphoserine, clone 4A4

(mouse monoclonal IgG₁)

Monoclonal Antibody

Cat. #05-1000

Lot # R0709B0209

pack size: 100 µg

FOR RESEARCH USE ONLY
NOT FOR USE IN HUMANS

Store at -20°C



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Applications	Species Cross-Reactivity	Antibody Isotype	Epitope/Region	Host Species	Molecular Weight	Genebank Accession#
WB, IF, FC, IH(P), ELISA	WR	IgG1	N/A	M	Varies	N/A

Background

The identification of protein phosphorylation as a regulatory mechanism originated from studies by Fischer and Krebs in the mid 1950s that later earned them the 1992 Nobel prize. It is the major mechanism for the regulation of diverse cellular processes including cell division, protein synthesis, transcriptional regulation and neurotransmission. The steady state phosphorylation of any given substrate is governed by the opposing activities of kinases and phosphatases. It is now believed that a third of all eukaryotic cellular proteins are phosphorylated and that the majority of all phosphorylation events occur on serine and threonine residues (>95%).

Presentation

100 µg of protein G purified mouse monoclonal IgG1 in 100 µL of PBS with 0.05% sodium azide and 30% glycerol.

Specificity

Serine-phosphorylated proteins from all species.

Immunogen

Phosphoserine coupled to KLH.

Method of Purification

Protein G-Sepharose chromatography.

Storage/Handling

2 years at -20°C from date of shipment.

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.

Molecular Weight

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

Control

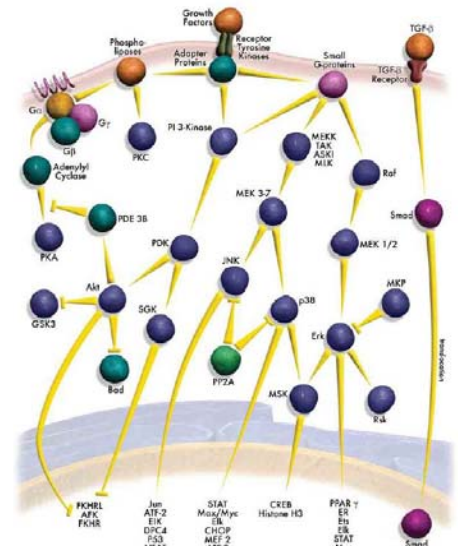
Included Positive Control: Cat.# 12-628, Calyculin A/Okadaic-treated A431 cell lysate is provided as a free positive control for western immunoblotting. Aliquot as desired, refreeze immediately, and store at -20°C. The lysate is stable for 6 months at -20°C.

Quality Control Testing

Routinely evaluated by immunoblot analysis on lysate from Calyculin A/Okadaic-treated human A431 carcinoma cells.

Immunoblot Analysis

0.5–2 µg/mL of this lot detected serine-phosphorylated proteins in a lysate from either insulin or Calyculin A/Okadaic-treated human A431 carcinoma cells.



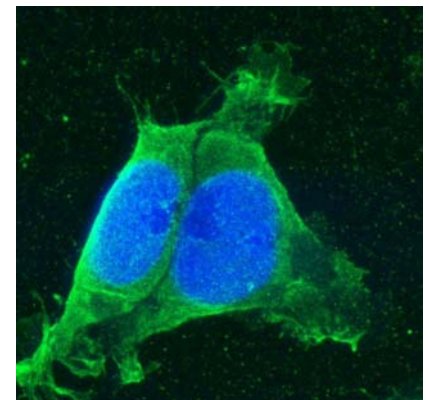
Immunoblot Analysis:

CalyculinA/Okadaic Acid untreated or treated A431 lysate (lanes 1 & 2, respectively) or Insulin untreated or treated 293 cell lysate (lanes 3 & 4, respectively) were resolved by electrophoresis, transferred to PVDF, and probed with anti-phosphoserine,4A4 (0.5 µg/mL). Proteins were visualized using a goat anti-mouse secondary antibody conjugated to HRP and a chemiluminescence detection system.

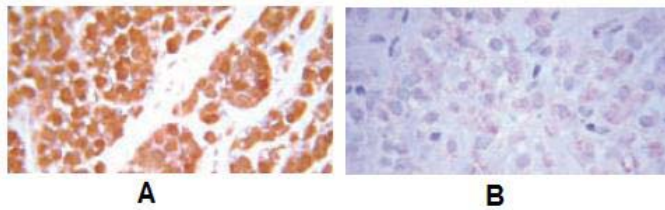
References

1. Bayascas, JR and Alessi, DR (2005) Mol. Cell 18:2 :143-145.
2. Chiang, GG and Abraham, RT (2005). J. Biol. Chem. 280(27): 25485-90.

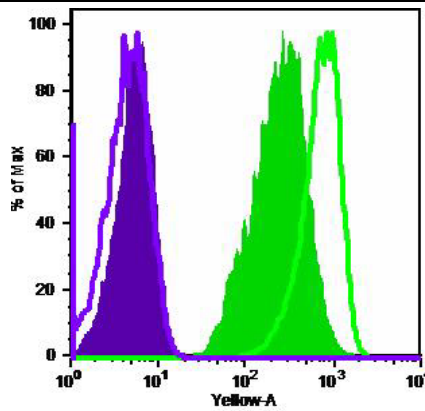
Immunofluorescence:



Confocal immunofluorescence image of insulin-treated 293 cells labeled with Anti-phosphoserine, clone 4A4 (green) and DAPI (blue).



Immunohistochemistry: Untreated and phosphorylated serine pre-treated (panels A and B, respectively) 4A4 staining pattern/morphology on paraffin embedded human breast cancer. Tissue pre-treated with citrate buffer pH 6.0. Antibody diluted to 1:500, IHC HRP/DAB detection system.



Flow Cytometry: Flow cytometry analysis using anti-phosphoserine, clone 4A4. Cells were either untreated (shaded, green) or treated with Calyculin A/Okadaic acid (30 minutes) (unshaded, green). Analysis was run with mouse IgG1 control (purple).

PROTOCOLS

Immunoblot

*NOTE: 4A4 can be used with either BSA or milk for the blocking and primary incubation steps of western blot, but either **REQUIRES** milk to be used for secondary antibody incubation. Milk is preferred as it gives cleaner results, but requires the use of more antibody (2-4 µg/mL), but can result in possible loss of certain harder to detect serine phosphorylated substrates.

1. Perform SDS-PAGE on a cell lysate sample and transfer the proteins to PVDF. Wash the blotted PVDF with TBST.
2. Block the blotted PVDF in freshly prepared TBST with either 5% BSA or 3% nonfat dry milk (Catalog # 20-200) for 60 minutes at room temperature with constant agitation.
3. Incubate the blocked PVDF with anti-Phosphoserine, clone 4A4 diluted to 0.5 µg/mL in TBST/BSA or 2 µg/mL in TBST/Milk with agitation for 1 hour at room temperature or overnight at 4°C.
4. Wash the PVDF three times with TBST.
5. Incubate the PVDF in the secondary reagent of choice (a goat anti-mouse HRP conjugated, Catalog # 12-349, 1:4000 dilution, was used) in TBST/Milk for 1 hour at room temperature with agitation.
6. Wash the PVDF TBS-0.05% Tween®-20 4 times for 3-5 minutes.
7. Use detection method of choice (enhanced chemiluminescence with a 30 second exposure was used).

Immunofluorescence Microscopy

1. Grow cells with appropriate treatment, aspirate media off, wash, and fix for 10 minutes with 3.7% paraformaldehyde in PBS for 20 minutes.
 2. Wash 3 times in PBST
 3. Permeabilize cells with 0.3% NP40 or Triton-X 100 in PBS for 5 minutes at room temperature.
 4. Wash 2 times with PBST
 5. Block cells with blocking buffer (PBST with 5%BSA) for 1 hour at room temperature.
 6. Incubate cells with 10 µg/mL anti-phosphoserine, clone 4A4 in 5% BSA in PBST for 2 hours at room temperature.
 7. Wash cells 3 times with PBST
 8. Incubate cells with diluted fluorescently-conjugated anti-mouse antibody in PBST for 30-45 minutes at room temperature.
 9. Wash cells 3 times with PBST.
 10. Mount slides with medium for fluorescent staining.
- * Store sample in the dark

Immunohistochemistry

1. Pre-treat with citrate buffer pH 6.0 for 20 minutes (HIER: Heat Induced Epitope Retrieval).
2. Follow protocol in cat. No. DAB150

RELATED PRODUCTS (specific)

cat #	description
05-1000X	Anti-Phosphoserine, clone 4A4, 50µg
05-1000MG	Anti-Phosphoserine, clone 4A4, 1MG
17-499	Phospho Explore pack (05-1000X and 05-1050X)
05-1050	Anti-Phosphotyrosine, 4G10 Platinum
05-1050X	Anti-Phosphotyrosine, 4G10 Platinum
05-1050ML	Anti-Phosphotyrosine, 4G10 Platinum
16-204	Anti-Phosphotyrosine, recombinant clone 4G10®, biotin conjugate
16-184	Anti-Phosphotyrosine, recombinant clone 4G10®, HRP conjugate
16-199	Anti-Phosphotyrosine, recombinant clone 4G10®, agarose conjugate
05-321	Anti-Phosphotyrosine, clone 4G10®
05-321MG	Anti-Phosphotyrosine, clone 4G10®
05-321X	Anti-Phosphotyrosine, clone 4G10®
16-101	Anti-Phosphotyrosine, clone 4G10®, agarose conjugate
16-104	Anti-Phosphotyrosine, clone 4G10®, FITC conjugate
16-105	Anti-Phosphotyrosine, clone 4G10®, HRP conjugate

RELATED PRODUCTS (non-specific)

cat #	description
IPVH00010	Immobilon-P 26.5 cm x 3.75 m Roll PVDF 0.45 um
IPFL00010	Immobilon-FL 26.5 cm x 3.75 m Roll PVDF 0.45 um
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 um
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
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® products biotools cell culture enzymes kits proteins/peptides siRNA/cDNA products

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