

Anti-Phosphoserine, clone 4A4 (mouse monoclonal IgG1)

Monoclonal Antibody

Cat. # 05-1000

Lot # DAM1597367

pack size: 100 µg

Store at -20°C

FOR RESEARCH USE ONLY
NOT FOR USE IN HUMANS



Certificate of Analysis

page 1 of 2

Applications	Species Cross-Reactivity	Antibody Isotype	Epitope/Region	Host Species	Molecular Weight	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

Purified mouse monoclonal IgG1 in buffer containing PBS with 0.1% sodium azide and 30% glycerol.

Concentration

1 mg/mL

Specificity

Serine-phosphorylated proteins from all species

Immunogen

Phosphoserine coupled to KLH

Molecular Weight

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

Method of Purification

Protein G-Sepharose chromatography

Storage and Handling

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

For maximum recovery, centrifuge the original vial prior to cap removal. If the product has accidentally been frozen and thawed, spin it at 13,000 x g for 10 minutes at 2-8°C.

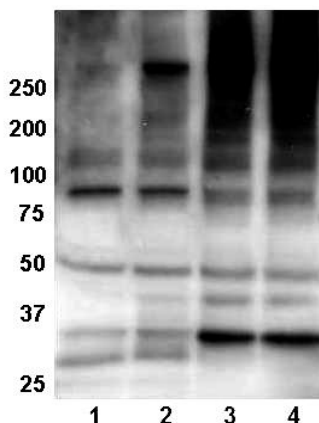
Control

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

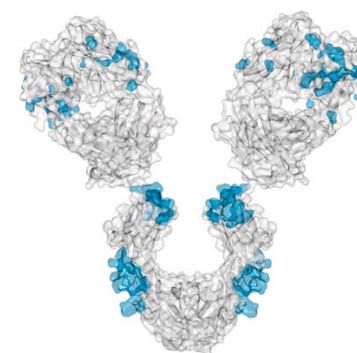
Routinely evaluated by Western Blot analysis on lysate from Calyculin A/Okadaic-treated human A431 carcinoma cells.

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



Western Blot Analysis:

Calyculin A/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 antiphosphoserine, 4A4 (0.5 µg/mL). Proteins were visualized using a goat anti-mouse secondary antibody conjugated to HRP and a chemiluminescence detection system.

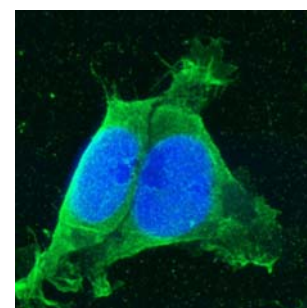


References

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.

Additional Research Applications

Immunofluorescence:



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

APPLICATION LEGEND: WB Western Blotting FC Flow Cytometry (FACS) ELISA Enzyme-linked Immunosorbent Assay

IP Immunoprecipitation IC Immunocytochemistry IF Immunofluorescence IH(P) Immunohistochemistry (Paraffin)

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

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

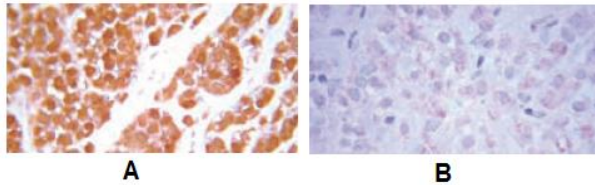
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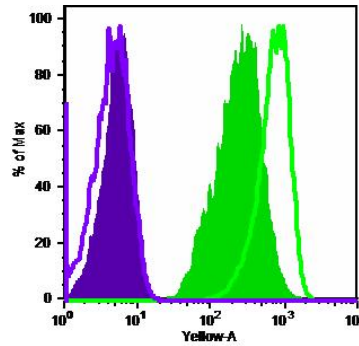
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Additional Research Applications

ELISA: Recommended



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, of a previous lot, 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/Okadain acid (30 minutes) (unshaded, green). Analysis was run with mouse IgG1 control (purple).

PROTOCOLS

Western Blot

*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, 50mg
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
12-349	■ Goat Anti-Mouse 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 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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