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AM00117PU-N Monoclonal Antibody to Phosphoserine (incl. pos. control) - Purified

Quantity: 0.1 mg

Background: Phosphorylation and dephosphorylation of cellular proteins are central steps in transducing extracellular signals to the cell nucleus. Phosphorylated epitopes may serve as docking sites for the assembly of protein complexes or may alter the 3-dimensional protein structure thus modulating enzymatic activity or the ability to undergo protein-protein-interactions. Modification of proteins on serine residues is mediated by serine/threonine kinases.
Please note that phosphoserine detection by monoclonal antibodies is always dependent on the surrounding amino acid sequence!

Host / Isotype: Mouse / IgM

Recommended Isotype Controls: SM13P

Clone: 16B4

Immunogen: Phosphopeptide conjugated to KLH.
Remarks: Epitope:...pSer-Pro...;...pSer-Lys

Format: **State:** Lyophilized purified Ig fraction
Purification: Size Exclusion Chromatography.
Buffer System: 1 ml 2x PBS containing 0.09% Sodium Azide, PEG and Sucrose
Reconstitution: Restore with 1.0 ml H2O (15 min, RT).

Applications: **Western Blot**: 1 µg/ml for HRPO/ECL detection.
Recommended blocking buffer: BSA/Tween 20 based blocking buffer. **DO NOT USE MILK OR CASEIN FOR BLOCKING!**
ELISA: 0.05 µg/ml.
Immunoprecipitation: 1-10 µg per 10e6 pervanadate-treated A431 cells.
Included Positive Control: Phosphoserine/Phosphothreonine (for details see "**Protocols**").
Other applications not tested. Optimal dilutions are dependent on conditions and should be determined by the user.

Specificity: AM00117PU-N recognizes a broad range of serine-phosphorylated proteins in crude cell extracts, preferring positively charged amino acids directly neighboured to phosphoserine.
Species: Human, Mouse, Rat and Dog.
Other species not tested.

Storage: Store lyophilized (preferably in a desiccator) at -20°C and reconstituted (aliquote and freeze in liquid nitrogen) at -80°C.
Avoid repeated freezing and thawing.
Thaw aliquots at 37°C. Thawed aliquots may be stored at 4°C up to 1 week.
Shelf life: one year from despatch.

General Readings:

1. Tisdale EJ. Glyceraldehyde-3-phosphate dehydrogenase is phosphorylated by protein kinase Ciota /lambda and plays a role in microtubule dynamics in the early secretory pathway. J Biol Chem. 2002 Feb 1;277(5):3334-41. Epub 2001 Nov 27. PubMed PMID: 11724794.
2. Alvarado-Kristensson M, Andersson T. Protein phosphatase 2A regulates apoptosis in neutrophils by dephosphorylating both p38 MAPK and its substrate caspase 3. J Biol Chem. 2005 Feb 18;280(7):6238-44. Epub 2004 Nov 29. PubMed PMID: 15569672.
3. Alvarado-Kristensson M, Melander F, Leandersson K, Rönnstrand L, Wernstedt C, Andersson T. p38-MAPK signals survival by phosphorylation of caspase-8 and caspase-3 in human neutrophils. J Exp Med. 2004 Feb 16;199(4):449-58. PubMed PMID: 14970175.
4. Site-specific Srb10-dependent phosphorylation of the yeast mediator subunit Med2 regulates gene expression from the 2-µm plasmid. M. Hallberg, et al.; PNAS. 101, 3370 (2003).
5. LaFevre-Bernt MA, Ellerby LM. Kennedy's disease. Phosphorylation of the polyglutamine-expanded form of androgen receptor regulates its cleavage by caspase-3 and enhances cell death. J Biol Chem. 2003 Sep 12;278(37):34918-24. Epub 2003 Jun 24. PubMed PMID: 12824190.
6. Martelli AM, Billi AM, Manzoli L, Faenza I, Aluigi M, Falconi M, et al. Insulin selectively stimulates nuclear phosphoinositide-specific phospholipase C (PI-PLC) beta1 activity through a mitogen-activated protein (MAP) kinase-dependent serine phosphorylation. FEBS Lett. 2000 Dec 15;486(3):230-6. PubMed PMID: 11119709.
7. Ducret C, Maira SM, Lutz Y, Wasylyk B. The ternary complex factor Net contains two distinct elements that mediate different responses to MAP kinase signalling cascades. Oncogene. 2000 Oct 19;19(44):5063-72. PubMed PMID: 11042694.
8. Müller G, Rouveyre N, Upshon C, Bandlow W. Insulin signaling in the yeast Saccharomyces cerevisiae. 3. Induction of protein phosphorylation by human insulin. Biochemistry. 1998 Jun 16;37(24):8705-13. PubMed PMID: 9628732.
9. Bendt AK, Burkovski A, Schaffer S, Bott M, Farwick M, Hermann T. Towards a phosphoproteome map of Corynebacterium glutamicum. Proteomics. 2003 Aug;3(8):1637-46. PubMed PMID: 12923788.
10. Trockenbacher A, Suckow V, Foerster J, Winter J, Krauss S, Ropers HH, et al. MID1, mutated in Opitz syndrome, encodes an ubiquitin ligase that targets phosphatase 2A for degradation. Nat Genet. 2001 Nov;29(3):287-94. PubMed PMID: 11685209.
11. Suer S, Sickmann A, Meyer HE, Herberg FW, Heilmeyer LM. Human phosphatidylinositol 4-kinase isoform PI4K92. Expression of the recombinant enzyme and determination of multiple phosphorylation sites. Eur J Biochem. 2001 Apr;268(7):2099-106. PubMed PMID: 11277933.

Protocols:

Positive control: pSer / pThr Molecular Weight Marker

Formulation:
The pSer/pThr molecular weight marker contains rabbit muscle phosphoproteins isolated by Fe3+/IDA - affinity chromatography. Proteins are lyophilized from PBS/NaF/PEG/Sucrose/ Bromophenolblue and Na - azide. After reconstitution the solution contains 0.09% Na-azide.

Stability:
Reconstitute by addition of 200 µl H2O. After complete solubilization add 200 µl 2x SDS-PAGE sample buffer, mix and incubate at 90°C for 5 min.

Application:
The pSer/pThr molecular weight marker is recommended for immunoblot applications. Use 20µl molecular weight marker per lane. Note: Use BSA based blot incubation buffers. Milk, Casein and Blotto might interfere with antibody - antigen interaction.

Storage:
Aliquote and store frozen.
Avoid repeated freeze/thaw cycles.
Shelf life: one year from despatch.

Pictures:

Phosphoserine Detection:
Phosphoprotein Positive Control was probed with: Lane 1: mab 1C8 (IgM), 1 µg/ml Lane 2: mab 4A3 (IgM), 1 µg/ml Lane 3: mab 4A9 (IgM), 1 µg/ml Lane 4: mab 4H4 (IgM), 1 µg/ml Lane 5: mab 7F12 (IgG), 1 µg/ml Lane 6: mab 16B4 (IgM), 1 µg/ml