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AM00113PU-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.
Host / Isotype:	Mouse / IgM
Recommended Isotype Controls:	SM13P
Clone:	4A3
Immunogen:	Synthetic phosphopeptide conjugated to KLH.
Format:	**State:** Lyophilized purified Ig fraction. **Purification:** Subsequent Thiophilic Adsorption and Size Exclusion Chromatography. **Buffer System:** 1 ml 2 x PBS containing 0.09% Sodium Azide, PEG and Sucrose. **Reconstitution:** Restore with 1.0 ml H2O (15 min, RT).
Applications:	**ELISA**: Use at 0.05 µg/ml. **Immunoprecipitation**: Use at 1-10 µg per 10e6 pervanadate-treated A431 cells. **Western Blot (Immunoblotting)**: 1 µg/ml for HRPO/ECL detection. Recommended blocking buffer: BSA/Tween 20 based blocking buffer. **DO NOT USE MILK OR CASEIN FOR BLOCKING!** Other applications not tested. Optimal dilutions are dependent on conditions and should be determined by the user.
Specificity:	This antibody recognizes a broad range of serine-phosphorylated proteins in crude cell extracts, preferring positively charged amino acids directly neighboured to phosphoserine. Please note that phosphoserine detection by monoclonal antibodies is always dependent on the surrounding amino acid sequence! **Species:** Human, Mouse, Rat and Dog. Other species not tested.
Add. Information:	This product contains a Positive Control (for details see **"Protocols"**).
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 2-8°C up to 1 week. Shelf life: one year from despatch.

General Readings:

1. Diversity of Regulatory Phosphorylation of the Na+/K+ - ATPase from Mammalian Kidneys and Xenopus Oocytes by Protein Kinases: Characterization of the Phosphorylation Site for Protein Kinase C. L. A. Vasilets; Cell Physiol. Biochem. 7, 1 (1997).
2. Brüstle B, Kreissl S, Mykles DL, Rathmayer W. The neuropeptide proctolin induces phosphorylation of a 30 kDa protein associated with the thin filament in crustacean muscle. J Exp Biol. 2001 Aug;204(Pt 15):2627-35. PubMed PMID: 11533112.
3. 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.
4. 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.
5. Frick W, Bauer A, Bauer J, Wied S, Müller G. Insulin-mimetic signalling of synthetic phosphoinositolglycans in isolated rat adipocytes. Biochem J. 1998 Nov 15;336 (Pt 1):163-81. PubMed PMID: 9806898.

Protocols:

Positive Control: pSer / pThr Molecular Weight Marker

Formulation

The pSer/pThr molecular weight marker contains rabbit muscle phosphoproteins isolated by Fe+/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.

Application

The pSer/pThr molecular weight marker is recommendede for immunoblot applications. Use 20 µl molecular meight 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 freezing and thawing.
Shelf life: one year from despatch.

Pictures:

Figure 1. 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