Rabbit (polyclonal) Anti-Smooth Muscle Myosin Light Chain Kinase [pS^{1760}] Phosphospecific Antibody, Unconjugated

PRODUCT ANALYSIS SHEET

Catalog Number:	441085G (10 mini-blot size)
Lot Number:	See product label
Volume:	100 µL
Form of Antibody:	Rabbit polyclonal immunoglobulin in Dulbecco's phosphate buffered saline (without Mg^{2+} and Ca^{2+}), pH 7.3 (+/- 0.1), 50% glycerol with 1.0 mg/mL BSA (IgG, protease free) as a carrier.
Preservative:	0.05% sodium azide (Caution: sodium azide is a poisonous and hazardous substance. Handle with care and dispose of properly.)
Purification:	Purified from rabbit serum by sequential epitope-specific chromatography. The antibody has been negatively preadsorbed using a non-phosphopeptide corresponding to the site of phosphorylation to remove antibody that is reactive with non-phosphorylated Myosin Light Chain Kinase (MLCK). The final product is generated by affinity chromatography using a MLCK-derived peptide that is phosphorylated at serine 1760.
Immunogen:	The antiserum was produced against a chemically synthesized phosphopeptide derived from the region of human myosin light chain kinase (MLCK) that contains serine 1760. The sequence is conserved in human, mouse and rat.
Target Summary:	Smooth Muscle Myosin Light Chain Kinase (MLCK) is a multifunctional regulatory protein of smooth muscle contraction (SMC), and a key element in ligand-mediated endothelial cell gap formation and vascular permeability, motility and morphology. Smooth muscle MLCK exists in at least two isoforms, short (~150 kDa) and long (210 kDa) which are identical except for an extended amino terminus with two additional putative actin-binding motifs in the long isoform. MLCK is phosphorylated by several kinases including protein kinase A (PKA), and mediates its function by phosphorylating 20 kDa myosin light chain (MLC20). Phosphorylation of MLCK inhibits the actin-activated ATPase of myosin II by reducing its affinity for actin. MLCK possesses a counter-balancing role in vascular regulation, by mediating vasoconstriction via direct action on SMCs and vasodilation via action on endothelial cells (ECs).
Reactivity:	Rabbit, mouse and bovine MLCK. Human MLCK (100% homologous) has not been tested, but is expected to react.
Applications:	The antibody has been used in Western blotting. Other applications may work but have not been tested.
Suggested Working Dilutions:	For Western blotting applications, we recommend using the antibody at a 1:1000 starting dilution. The optimal antibody concentration should be determined empirically for each specific application.
Storage:	Store at –20°C. We recommend a brief centrifugation before opening to settle vial contents. Then, apportion into working aliquots and store at –20°C. For shipment or short-term storage (up to one week), 2-8°C is sufficient.
Expiration Date:	Expires one year from date of receipt when stored as instructed.

This product is for research use only. Not for use in diagnostic procedures.

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Positive Control Used: KCl-treated rabbit muscle extracts; COS cells over-expressing rabbit smooth muscle short MLCK, phosphorylated *in vitro* by PKA.

Related Products:

Antibodies:

PAK1/2/3 [pS^{141}], Cat. # 44940G

PAK1/2/3 [pT^{423}], Cat. # 44942G

MLC [pS^{19}], Cat. # 44260G

MAPKAP-K2 [pT^{334}], Cat. # 44516G

RSK1 [pS^{221}] /2 [pS^{227}], Cat. # 44924G

PKA catalytic α/β [pT^{197}], Cat. # 44988

PKA regulatory II β [pS^{114}], Cat. # 44998G

Rac-1/cdc42 [pS^{71}], Cat. # 44214G

LIMK1/2 [pTpY^{507}/ ^{508}], Cat. # 441076G

Cofilin [pS^{3}], Cat. # 441072G

CaMKIIα [pT^{286}], Cat. # 44674G

References:

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Takaishi, K., et al. (2000) Localization and activity of myosin light chain kinase isoforms during the cell cycle. J. Cell. Biol. 151(3):697-708.

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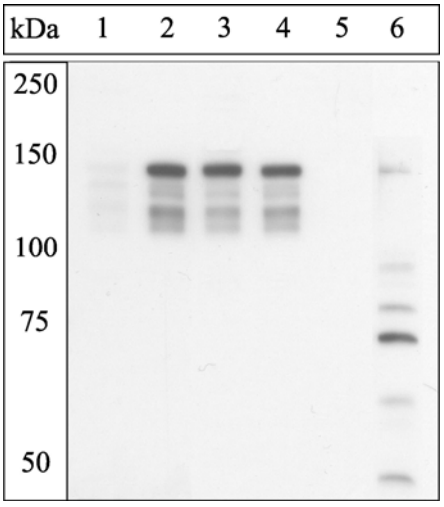

Up-regulation, Antibody-Peptide Competition and Phosphatase Treatment

Extracts of COS cells transfected with muscle MLCK unstimulated (1) or phosphorylated *in vitro* by 0.05 µg of PKA (per µg of extract) for 10 minutes at 37°C (2-6), were resolved by SDS-PAGE on a 4-12% Tris-glycine gel and transferred to PVDF. The membrane was left untreated (1-5) or treated with lambda phophatase (6), blocked with a 5% BSA-TBST buffer for one hour at room temperature, and then incubated with the MLCK [pS^{1760}] antibody for two hours at room temperature in 3% BSA-TBST buffer, following prior incubation with: no peptide (1, 2, 6), the non-phosphopeptide corresponding to the phosphopeptide immunogen (3), a generic phosphoserine-containing peptide (4), or the phosphopeptide immunogen (5). After washing, the membrane was incubated with goat F(ab')$_2$ anti-rabbit IgG HRP conjugate (Cat. # ALI4404) and signals were detected using the Pierce SuperSignal™ method.

The data show that PKA induces the phosphorylation of MLCK at serine 1760 in this cell system. The data also show that only the phosphopeptide corresponding to MLCK [pS^{1760}] blocks the signal and that phosphatase stripping eliminates the signal, verifying that the antibody is indeed phosphorylation site-specific.

(The transfected cell lysates were a generous gift from Dr. James Stull [UTSW]).

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Western Blotting Procedure

1. Lyse approximately 10^7 cells in 0.5 mL of ice cold Cell Lysis Buffer (formulation provided below). This buffer, a modified RIPA buffer, is suitable for recovery of most proteins, including membrane receptors, cytoskeletal-associated proteins, and soluble proteins. This cell lysis buffer formulation is available as a separate product which requires supplementation with protease inhibitors immediately prior to use (Invitrogen cat. # FNN0011). Other cell lysis buffer formulations, such as Laemmli sample buffer and Triton-X 100 buffer, are also compatible with this procedure. Additional optimization of the cell stimulation protocol and cell lysis procedure may be required for each specific application.
2. Remove the cellular debris by centrifuging the lysates at 14,000 x g for 10 minutes. Alternatively, lysates may be ultracentrifuged at 100,000 x g for 30 minutes for greater clarification.
3. Carefully decant the clarified cell lysates into clean tubes and determine the protein concentration using a suitable method, such as the Bradford assay. Polypropylene tubes are recommended for storing cell lysates.
4. React an aliquot of the lysate with an equal volume of 2x Laemmli Sample Buffer (125 mM Tris, pH 6.8, 10% glycerol, 10% SDS, 0.006% bromophenol blue, and 130 mM dithiothreitol [DTT]) and boil the mixture for 90 seconds at 100°C.
5. Load 10-30 µg of the cell lysate into the wells of an appropriate single percentage or gradient minigel and resolve the proteins by SDS-PAGE.
6. In preparation for the Western transfer, cut a piece of PVDF membrane slightly larger than the gel. Soak the membrane in methanol for 1 minute, then rinse with ddH_2O for 5 minutes. Alternatively, nitrocellulose may be used.
7. Soak the PVDF membrane, 2 pieces of Whatman paper, and Western apparatus sponges in transfer buffer (formulation provided below) for 2 minutes.
8. Assemble the gel and membrane into the sandwich apparatus.
9. Transfer the proteins at 140 mA for 60-90 minutes at room temperature.
10. Following the transfer, rinse the membrane with Tris buffered saline for 2 minutes.
11. Block the membrane with blocking buffer (formulation provided below) for one hour at room temperature or overnight at 4°C.
12. Incubate the blocked blot with primary antibody at a 1:1000 starting dilution in Tris buffered saline supplemented with 3% Ig-free BSA and 0.1% Tween 20 for two hours at room temperature or overnight at 4°C.
13. Wash the blot with several changes of Tris buffered saline supplemented with 0.1% Tween 20.
14. Detect the antibody band using an appropriate secondary antibody, such as goat $F(ab')_2$ anti-rabbit IgG alkaline phosphatase conjugate (Cat. # ALI4405) or goat $F(ab')_2$ anti-rabbit IgG horseradish peroxidase conjugate (Cat. # ALI4404) in conjunction with your chemiluminescence reagents and instrumentation.

Cell Lysis Buffer Formulation:
10 mM Tris, pH 7.4
100 mM NaCl
1 mM EDTA
1 mM EGTA
1 mM NaF
20 mM $Na_4P_2O_7$
2 mM Na_3VO_4
0.1% SDS
0.5% sodium deoxycholate
1% Triton-X 100
10% glycerol
1 mM PMSF (made from a 0.3 M stock in DMSO) or 1 mM AEBSF (water soluble version of PMSF)
60 µg/mL aprotinin
10 µg/mL leupeptin
1 µg/mL pepstatin
(alternatively, protease inhibitor cocktail such as Sigma Cat. # P2714 may be used)

Transfer Buffer Formulation:
2.4 gm Tris base
14.2 gm glycine
200 mL methanol
Q.S. to 1 liter, then add
1 mL 10% SDS.
Cool to 4°C prior to use.

Tris Buffered Saline Formulation:
20 mM Tris-HCl, pH 7.4
0.9% NaCl

Blocking Buffer Formulation:
100 mL Tris buffered saline
5 gm Ig-free BSA
0.1 mL Tween 20

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