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Polyclonal Antibody to Myosin light chain Kinase (MLCK) pSer1760 - Aff - Purified

Catalog No.: BP7133

Quantity: 0.1 ml

Background: 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).

Host: Rabbit

Immunogen: Chemically synthesized phosphopeptide derived from the region of human myosin light chain kinase (MLCK) that contains serine 1760
Remarks: The sequence is conserved in human, mouse and rat.

Format: **State:** Liquid Ig fraction
Purification: 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.
Buffer System: Dulbecco's phosphate buffered saline (without Mg2+ and Ca2+), pH 7.3 (+/- 0.1), 50% glycerol with 1.0 mg/mL BSA (IgG, protease free) as a carrier, containing 0.05 % sodium azide as preservative.

Applications: Western blot (1:1000 starting dilution).
Positive Control Used: KCl-treated rabbit muscle extracts; COS cells over-expressing rabbit smooth muscle short MLCK, phosphorylated in vitro by PKA.
Other applications not tested. Optimal dilutions are dependent on conditions and should be determined by the user.

Specificity: This antibody detects MLCK.
Species: Human, Mouse, Rabbit, Bovine.
Other species not tested.

Storage:

Store the antibody at -20 °C. Can be shipped at 2 - 8 °C.
Avoid repeated freezing and thawing.
Centrifuge vial before opening.
Shelf life: One year from despatch.

General Readings:

Isotani, E., et al. (2004) Real-time evaluation of myosin light chain kinase activation in smooth muscle tissues from a transgenic calmodulin-biosensor mouse. Proc. Nat'l. Acad. Sci. USA 101(16):6279-6284.
Dulyaninova, N.G. and A.R. Bresnick (2004) The long myosin light chain kinase is differentially phosphorylated during interphase and mitosis. Exp. Cell Res. 299(2):303-314.
Totsukawa, G., et al. (2004) Distinct roles of MLCK and ROCK in the regulation of membrane protrusions and focal adhesion dynamics during cell migration of fibroblasts. J. Cell. Biol. 164(3):427-439.
Tinsley, J.H., et al. (2004) Isoform-specific knockout of endothelial myosin light chain kinase: closing the gap on inflammatory lung disease. Trends Pharmacol. Sci. 25(2):64-66.
Kamm, K.E. and J.T. Stull (2001) Dedicated myosin light chain kinases with diverse cellular functions. J. Biol. Chem. 276(7):4527-4530.
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.
Watanabe, H., et al. (2001) Myosin light-chain kinase regulates endothelial calcium entry and endothelium-dependent vasodilation. FASEB J. 15(2):282-284.
Smith, L., et al. (2002) Properties of long myosin light chain kinase binding to F-actin in vitro and in vivo. J. Biol. Chem. 277(38):35597-35604.

Protocols:

Western Blotting Procedure

1. Lyse approximately 10e7 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. 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 ultracentrifugedat 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 ddH2O for 5 minutes. Alternatively, nitrocellulose may be used.
7. Soak the 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 or goat F(ab)2 anti-rabbit IgG horseradish peroxidase conjugate in conjunction with your chemiluminescence reagents and instrumentation.

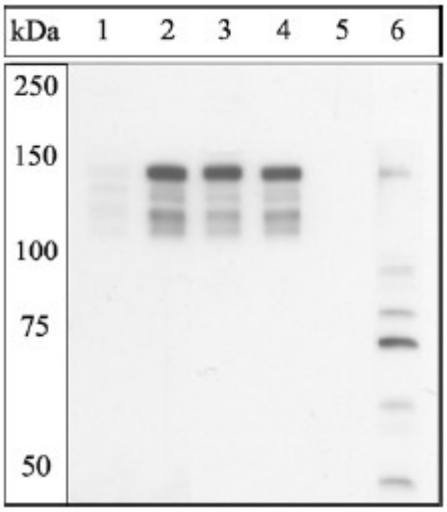
Cell Lysis Buffer Formulation:
10 mM Tris, pH 7.4
100 mM NaCl
1 mM EDTA
1 mM EGTA
1 mM NaF
20 mM Na4P2O7
2 mM Na3VO4
0.1% SDS
0.5% sodium deoxycholate
1% Triton-X 100
10% glycerol
1 mM PMSF (made from a 0.3 M stock in DMSO)
or 1 mM AEBSF (water soluble version of PMSF)
60 µg/mL aprotinin
10 µg/mL leupeptin
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 BSA
0.1 mL Tween 20

Pictures:



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 [pS1760] 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 and signals were detected using the Pierce SuperSignalTM 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 [pS1760] 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]).