Datasheet for 600-401-416S

Myosin phospho S19/phospho S20 Antibody

Overview

Description:	Anti-Myosin pS19/pS20 (RABBIT) Antibody - 600-401-416S
Item No.:	600-401-416S
Size:	25 µL
Applications:	ELISA, IHC, WB, IF, Multiplex
Reactivity:	Human, Mouse
Host Species:	Rabbit

Product Details

Background:	Myosin is the major component of thick muscle filaments, and is a long asymmetric molecule containing a globular head and a long tail. The molecule consists of two heavy chains each ~200,000 daltons, and four light chains each ~16,000 - 21,000 daltons. Activation of smooth and cardiac muscle primarily involves pathways that increase calcium levels and myosin phosphorylation, resulting in contraction. Myosin light chain phosphatase acts to regulate muscle contraction by dephosphorylating activated myosin light chain. This antibody is specific for the phosphorylated form of myosin light chain. The selected peptide sequence used to generate the polyclonal antibody is located near the amino terminal end of the polypeptide corresponding to the smooth/non-muscle form of myosin regulatory light chain found in cardiac myocytes in addition to smooth and non-muscle cells. This sequence differs from that of the sarcomeric/cardiac form of myosin regulatory light chain that has a different sequence around the phosphorylation site. Human and mouse have almost identical sequences. In human the phosphorylation site is pS19, while in mouse the site maps to pS20.
Synonyms:	rabbit anti-Myosin p19/pS20 antibody, Myosin regulatory light chain 12A, Myosin regulatory light chain MRLC3, Myosin regulatory light chain 2 nonsarcomeric, Myosin RLC, MLC-2B, HEL-S-24, Epididymis secretory protein Li 24, MLCB
Host Species:	Rabbit
Clonality:	Polyclonal
Format:	IgG

Target Details

Gene Name:	MYL12A

Reactivity:	Human, Mouse
PTM Specificity:	Phosphorylation
Immunogen Type:	Conjugated Peptide
Immunogen:	Human Myosin Light Chain phospho peptide corresponding to a region near the amino terminus of the human smooth/non-muscle form of myosin regulatory light chain conjugated to Keyhole Limpet Hemocyanin (KLH).
Purity/Specificity:	This affinity purified antibody is directed against the regulatory light chain of smooth and non-muscle myosin. The antibody is phosphospecific and detects monophosphorylated and diphosphorylated forms of the protein. The product was affinity purified from monospecific antiserum by immunoaffinity purification. Antiserum was first purified against the phosphorylated form of the immunizing peptide. The resultant affinity purified antibody was then cross-adsorbed against the non-phosphorylated form of the immunizing peptide. This phosphospecific polyclonal antibody is specific for the phosphorylated pS19/pS20 form of the protein, depending on the source origin of the protein. Reactivity with non-phosphorylated myosin light chain is less than 1% by ELISA. Cross reactivity is expected with myosin light chain from human and mouse. Reactivity with the protein from other species has not been determined. However, the sequence of the immunogen is nearly identical in mammalian and avian species. BLAST search analysis was used to determine that the smooth and non-muscle forms of myosin regulatory light chain have identical sequences. Cross reactivity is expected.
Relevant Links:	• UniProtKB - P19105 • NCBI - AAH16372.1 • GeneID - 10627

Application Details

Tested Applications:	ELISA, IHC, WB
Suggested Applications:	IF, Multiplex (Based on references)
Application Note:	This phospho specific polyclonal antibody was tested by ELISA, immunohistochemistry, and immunoblotting. Immunoblotting was used to show reactivity with unstimulated and stimulated cardiac myocytes. The antibody was also reactive with the phosphorylated form of the immunizing peptide and minimally reactive with the non-phosphorylated form of the immunizing peptide. Although not tested, this antibody is likely functional by immunoprecipitation.
Assay Dilutions:	All assays should be optimized by the user. Recommended dilutions (if any) may be listed below.
ELISA:	1:10,000 - 1:30,000
IHC:	2.5 µg/ml
IP:	1:100

WB:	1:1,000 - 1:5,000

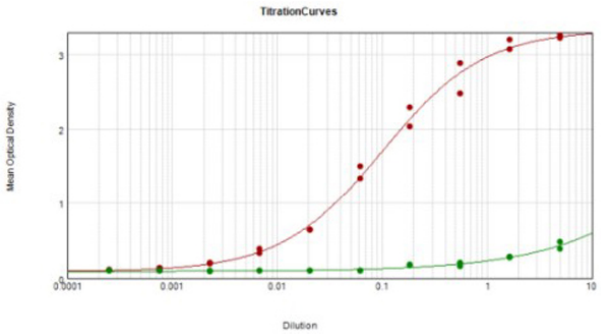
Formulation

Physical State:	Liquid (sterile filtered)
Concentration:	1.0 mg/mL by UV absorbance at 280 nm
Buffer:	0.02 M Potassium Phosphate, 0.15 M Sodium Chloride, pH 7.2
Preservative:	0.01% (w/v) Sodium Azide
Stabilizer:	None

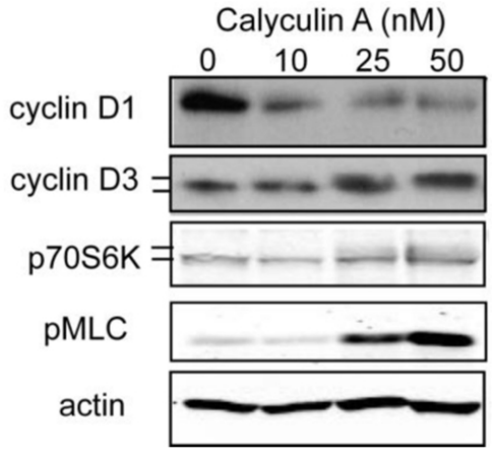
Shipping & Handling

Shipping Condition:	Dry Ice
Storage Condition:	Store vial at -20° C or below prior to opening. This vial contains a relatively low volume of reagent (25 µL). To minimize loss of volume dilute 1:10 by adding 225 µL of the buffer stated above directly to the vial. Recap, mix thoroughly and briefly centrifuge to collect the volume at the bottom of the vial. Use this intermediate dilution when calculating final dilutions as recommended below. Store the vial at -20°C or below after dilution. Avoid cycles of freezing and thawing.
Expiration:	Expiration date is one (1) year from date of receipt.

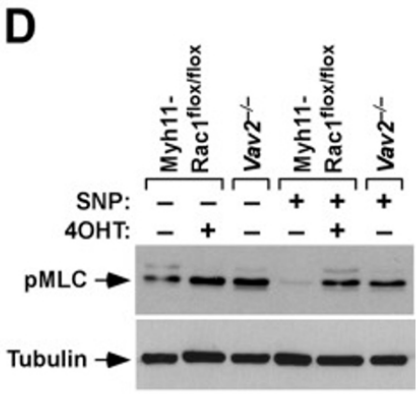
Images



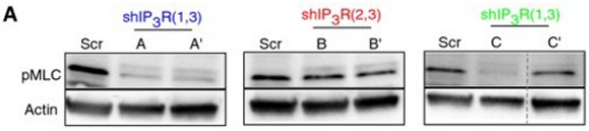
ELISA
ELISA Results of Rabbit Anti-Myosin pS19/pS20 Antibody tested against BSA-conjugated peptide of immunizing peptide. Each well was coated in duplicate with 0.1µg of Myosin pS19/pS20 [Red Line] and Myosin S19/S20 [Green Line]. The starting dilution of antibody was 5µg/ml and the X-axis represents the Log10 of a 3-fold dilution. This titration is a 4-parameter curve fit where the IC50 is defined as the titer of the antibody. Assay performed using Goat anti-Rabbit IgG Antibody Peroxidase Conjugated (Min X Bv Ch Gt GP Ham Hs Hu Ms Rt & Sh Serum Proteins) (p/n 611-103-122) and TMB ELISA Peroxidase Substrate (p/n TMBE-1000).



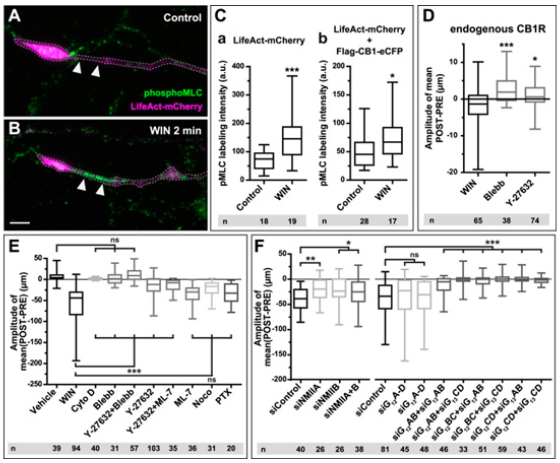
Western Blot
Effect of calyculin A on phosphorylation of endogenous proteins in MDA-MB-468 cells. MDA-MB-468 cells were treated with 10, 25, or 50 nM calyculin A for 30 min, processed as described in Materials and Methods and nitrocellulose membranes were blotted with antibodies against cyclin D1, total cyclin D3, total p70S6K, phospho myosin light chain (p/n 600-401-416), and actin. Phosphorylation of cyclin D3 and p70S6K results in slight reduction in mobility. Fig 2. PMID: 22069692



Western Blot
(D, upper) Representative immunoblot showing the amount of MLC phosphorylation in primary vSMCs from the indicated mice and culture conditions. (Lower) The amount of tubulin α was used as a loading control. Similar results were obtained in two additional independent experiments. Fig 4. PMID: 25288640

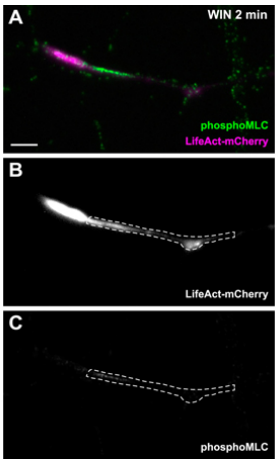


Western Blot
IP3R1 regulates myosin IIA activity and dynamics in migrating immature DCs.
A) Immunoblot showing the intracellular levels of phosphorylated MLC in DCs transduced with the shScramble or shRNAs for IP3Rs (shIP3R(1,3)A', shIP3R(2,3)B', and shIP3R (1,3)C'). Actin was used as a control.
Fig 5. PMID: 25637353

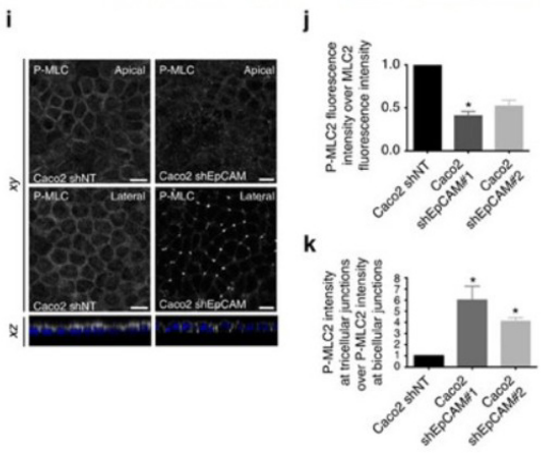


Immunofluorescence Microscopy

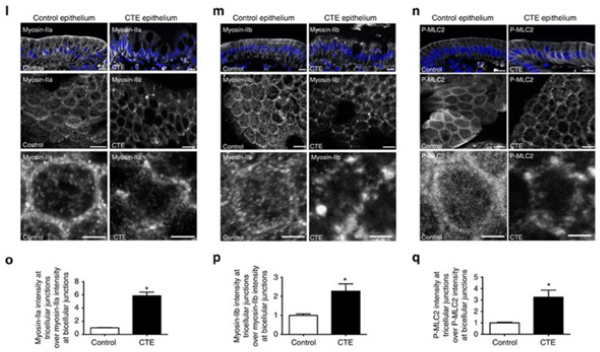
Cultured hippocampal neurons at DIV6 co-expressing a combination of LifeAct-mCherry, Flag-CB1R-eCFP, and EB3-eGFP as indicated and treated by WIN (100 nM) at 0 min. (A–B) Representative LifeAct-mCherry expressing growth cones (delimited with a dotted line) at 2 min after treatment with vehicle (A) or WIN (100 nM, B), labeled with a phospho-Myosin Light Chain (phosphoMLC) antibody. Arrowheads show the distal axon adjacent to the F-actin-rich growth cone where WIN induces rapid and strong upregulation of myosin light chain phosphorylation. (C) pMLC labeling intensity at the distal 50–60 µm of the axon, adjacent to the actin-rich growth cone, from neurons expressing LifeAct-mCherry (A) or co-expressing LifeAct-mCherry and Flag-CB1R-eCFP (B). The region-of-interest used to measure pMLC labeling intensity is delimited with a dotted line on a representative growth cone on Figure 3—figure supplement 1. (D) Amplitude of 100 nM WIN-induced growth cone retraction in neurons co-expressing LifeAct-mCherry and EB3-eGFP pre-treated with 25 µM blebbistatin or 10 µM Y-27632. (E) Amplitude of 100 nM WIN-induced growth cone retraction in neurons co-expressing LifeAct-mCherry, EB3-eGFP, and Flag-CB1R-eCFP pre-treated with: 1 µM cytochalasin D; 25 µM blebbistatin; 25 µM blebbistatin + 10 µM Y-27632; 10 µM Y-27632; 30 µM ML-7 + 10 µM Y-27632; 30 µM ML-7; 10 µM nocodazole; 100 ng/µl PTX. (F) Effect of siRNA-mediated knock-down of endogenous myosin IIA, IIB or of endogenous G12/G13 proteins on growth cone-retraction induced by 100 nM WIN in neurons co-expressing the three constructs, as compared to control (luciferase) siRNA. Results are pooled from at least two independent experiments, and outliers were removed in accordance with Grubb's test. Results in are expressed as boxplots. n.s $p > 0.05$; *$p < 0.05$; **$p < 0.01$; ***$p < 0.001$ calculated using Student's t-test on (C), Kruskal–Wallis one-way ANOVA followed by Dunn's post-tests on (D) and (E), and using one-way ANOVA followed by Newman–Keuls post-tests on (F). Scale bar: 10 µm. Fig 3. PMID: 25225054



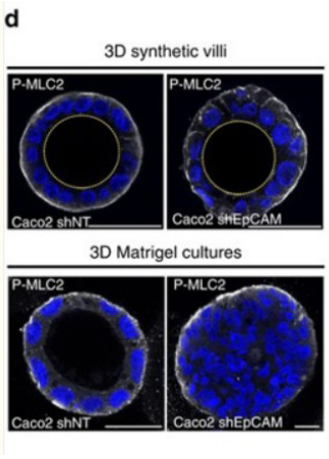
Immunofluorescence Microscopy
Another representative LifeAct-mCherry expressing growth cone 2 min after treatment with WIN (100 nM), labeled with the phosphoMLC antibody, similarly to Figure 3B. The region of interest used for the quantification of phosphoMLC labeling intensity, approximately 50–60 µm on each image, is marked with dotted lines. This region was delimited using the LifeAct-mCherry image (B) and quantified on the raw pMLC image (C). Scale bar: 10 µm.
Figure 3—figure supplement 1. PMID: 25225054



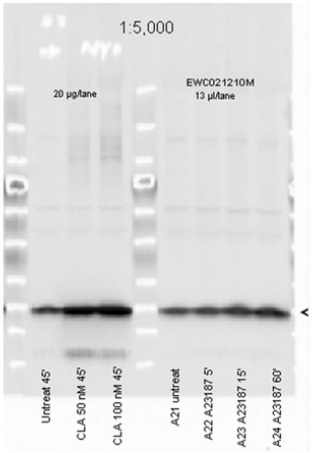
Immunofluorescence Microscopy
(i) Distribution of P-MLC2 in control or EpCAM-silenced cells. Scale bars, 5 µm. (j) Quantification of total P-MLC2 immunofluorescence intensity relative to MLC2 immunofluorescence intensity in control or EpCAM-depleted cells. One-way analysis of variance test with Dunnett's test, *P=0.007, **P=0.002. n(Caco2shNT)=30 cells, n (Caco2shEpCAM#1)=30, n(Caco2shEpCAM#2)=30. Caco2shNT=1, Caco2shEpCAM#1=0.4±0.05, Caco2shEpCAM#2=0.52±0.07. (k) Quantification of P-MLC2 immunofluorescence intensity at TCs relative to immunofluorescence intensity at bicellular junctions (BJs) in control or EpCAM-depleted cells. One-way analysis of variance with unpaired t-test, *P=0.0001. n(Caco2shNT)=30 cells, n(Caco2shEpCAM#1)=28, n(Caco2shEpCAM#2)=30. Caco2shNT=1, Caco2shEpCAM#1=6.01±1.24, Caco2shEpCAM#2=4.1±0.33. Fig 5. PMID: 28084299



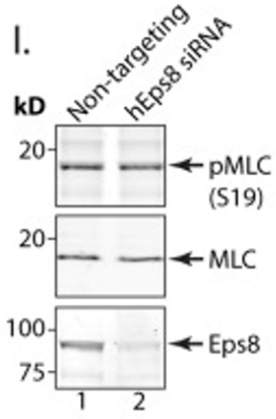
Immunofluorescence Microscopy
(l–n) Distribution of myosin-IIa, -IIb and P-MLC2 in control or CTE biopsies. Scale bars, 5 µm. N(Control)=3 biopsies, N (CTE)=3. (o–q) Quantification of myosin-IIa, -IIb or P-MLC2 immunofluorescence intensity at TCs relative to immunofluorescence intensity at BJs in control or CTE biopsies. N(Control)=3 biopsies, N(CTE)=3. Unpaired t-test, (o) *P<0.0001, (p) *P=0.0062, (q) *P=0.0016. n(Control myosin-IIa)=37 cells, n(CTE myosin-IIa)=29, n(Control myosin-IIb)=14, n(CTE myosin-IIb)=14, n(Control P-MLC2)=15, n(CTE P-MLC2)=21. Myosin-IIa(Control)=1±0.04, myosin-IIa(CTE)=5.86±0.54, myosin-IIb(Control)=1±0.08, myosin-IIb(CTE)=2.27±0.39, P-MLC2(Control)=1±0.07, P-MLC2(CTE)=3.25±0.62. For quantification, three independent replicates have been performed. Values are mean±s.e.m. Nuclei were stained with Hoechst 33342. Fig 5. PMID: 28084299



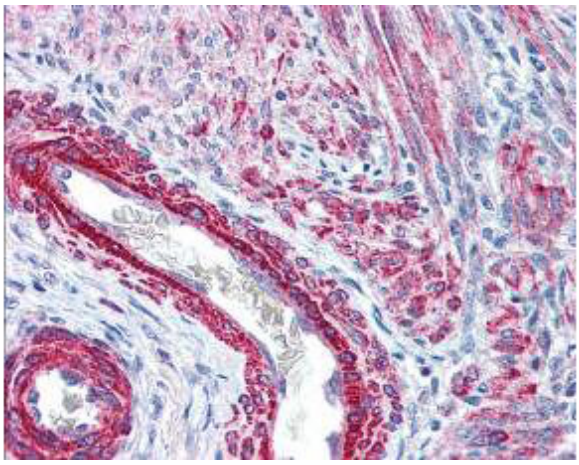
Immunofluorescence Microscopy
d) Confocal microscopy analysis of P-MLC2 distribution in control (Caco2 shNT) and EpCAM-silenced (Caco2 shEpCAM) cells that were grown on villous PDMS inserts or in 3D Matrigel cultures for 21 days. Transversal xy views are presented. Scale bars, 50 µm. Fig 9. PMID: 28084299



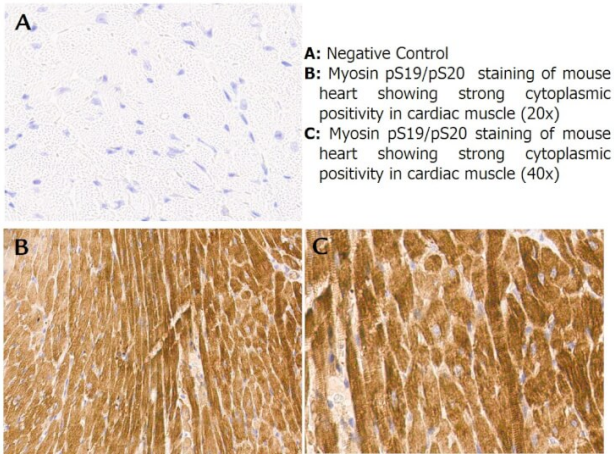
Western Blot
Affinity Purified Phospho specific antibody to Monophosphorylated Regulatory Light Chain of Smooth and Non-muscle Myosin at pS19/pS20 was used at a 1:5000 dilution to detect myosin light chain by Western blot. Either 13µL or 20 µg of a mouse cardiac myocyte lysate was loaded on a 4-20% Criterion gel for SDS-PAGE. Samples were either mock-treated or CLA-treated, as indicated. After washing, a 1:5,000 dilution of HRP conjugated Gt-a-Rabbit IgG (611-103 -122) preceded color development using Amersham's substrate system. Other detection methods will yield similar results. Data courtesy of the Alliance for Cellular Signaling (http://www.signaling-gateway.org).



Western Blot
I) Western blot analyses of Eps8, myosin II regulatory light chain (MLC p/n 600-401-938), and myosin II regulatory light chain phosphorylated on serine 19 (pMLC (S19) p/n 600-401-416) in lysates of A375 cells that were treated with non-targeting siRNAs (non-targeting) or siRNAs targeting human Eps8 to deplete Eps8 (hEps8 siRNA). Fig 2. PMID: 26163656

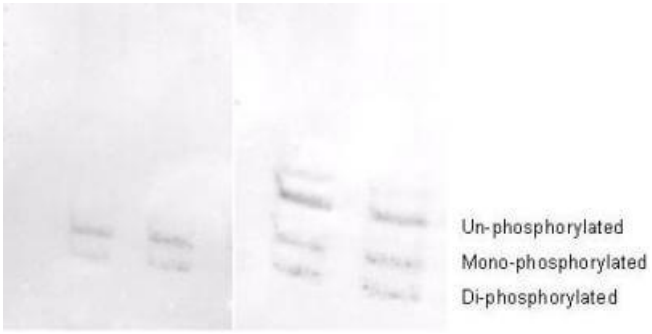


Immunohistochemistry
Rockland's affinity purified anti-Monophosphorylated RLC Smooth and Non-Muscle Myosin pS19/20 antibody was used at 2.5 µg/ml to detect signal in a variety of tissues including multi-human, multi-brain and multi-cancer slides. This image shows strong staining of both vascular and myometrial smooth muscle cells of the uterus. Tissue was formalin-fixed and paraffin embedded. The image shows localization of the antibody as the precipitated red signal, with a hematoxylin purple nuclear counterstain. Personal Communication, Tina Roush, LifeSpanBiosciences, Seattle, WA.

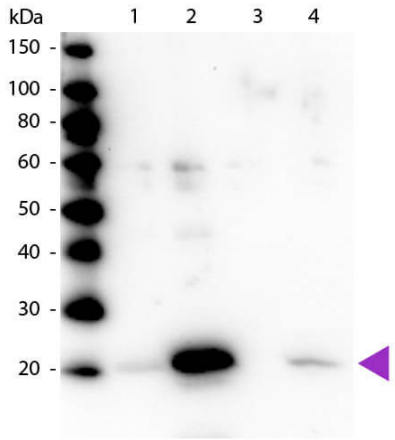


A: Negative Control
B: Myosin pS19/pS20 staining of mouse heart showing strong cytoplasmic positivity in cardiac muscle (20x)
C: Myosin pS19/pS20 staining of mouse heart showing strong cytoplasmic positivity in cardiac muscle (40x)

Immunohistochemistry
Immunohistochemistry with anti-myosin pS19/pS20 antibody showing strong cytoplasmic staining of myocytes in mouse heart muscle 20x and 40x (B & C). Staining was performed on Leica Bond system using the standard protocol. Formalin fixed/paraffin embedded tissue sections were subjected to antigen retrieval and then incubated with rabbit anti-myosin pS19/pS20 antibody at 1:100 dilution for 60 minutes. Biotinylated Anti-rabbit secondary antibody was used to detect primary antibody. The reaction was developed using streptavidin-HRP conjugated compact polymer system and visualized with chromogen substrate, 3’3-diamino-benzidine substrate (DAB). The sections were then counterstained with hematoxylin to detect cell nuclei.



Western Blot
Affinity purified phosphospecific antibody to phosphorylated regulatory light chain of smooth and non-muscle Myosin at pS19/pS20 was used at a 1:1000 dilution to detect myosin light chain by Western blot on 3T3 cell lysates. A standard urea/glycerol gel without SDS was used to separate phospho forms of regulatory light chain according to mass to charge ratios. In Panel A on the left, reactivity of Rockland's phosphospecific antibody is shown. In Panel B on the right, reactivity of commercially available pan reactive antibody that detects both un-phosphorylated and phosphorylated forms of regulatory light chain is shown. Rockland's phosphospecific antibody detects both mono-phosphorylated (pSer20 Mono-P-RLC) and di-phosphorylated (pThr19-pSer20 Di-P-RLC) regulatory light chain. Personal communication. J. Stull. UT Southwestern Medical Center.



Western Blot
Western blot of Rabbit Anti-Myosin pS19/pS20 primary antibody. Lane 1: Regulatory Light Chain Non-Phospho recombinant protein. Lane 2: Regulatory Light Chain Phospho recombinant protein. Lane 3: Smooth Muscle Non-Phospho recombinant protein. Lane 4: Smooth Muscle Phospho recombinant protein. Load: 50 ng per lane. Primary antibody: Myosin pS19/pS20 primary antibody at 1:1,000 overnight at 4°C. Secondary antibody: Peroxidase rabbit secondary antibody at 1:40,000 for 60 min at RT. Blocking: MB-070 for 30 min at RT. Predicted/Observed size: 20 kDa, 20 kDa for Regulatory Light Chain Phospho. Other band(s): None.

References

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