ROCKLAND
Immunochemicals for Research

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Certificate of Analysis

Product: Affinity Purified Anti-Regulatory Light Chain of Smooth and Non-muscle Myosin [Rabbit]

Code: 600-401-938 **Lot #** 17771

Size: 100 µg **Physical State:** Liquid (sterile filtered)

Antibody Concentration: 1.1 mg/ml (by UV absorbance at 280 nm)

Buffer: 0.02 M Potassium Phosphate, 0.15 M Sodium Chloride, pH 7.2

Stabilizer: None **Preservative:** 0.01% (w/v) Sodium Azide

Storage Conditions: Store vial at -20° C prior to opening. Centrifuge product if not completely clear after standing at room temperature. Dilute only prior to immediate use. For extended storage aliquot contents and freeze at -20° C or below. Avoid cycles of freezing and thawing. Expiration date is one (1) year from date of opening.

Background Information: 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 and myosin phosphorylation resulting in contraction. Myosin light chain phosphatase acts to regulate muscle contraction by dephosphorylating activated 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, mouse and rat have almost identical sequences.

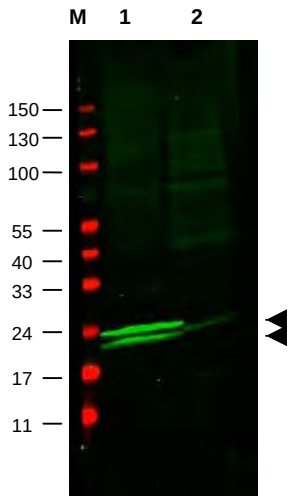

Figure. Western blot using ROCKLAND's anti-RLC of Smooth and Non-muscle Myosin antibody to detect vascular myosin (rat aorta, lane 1) but not cardiac myosin (mouse heart, lane2). Each lane was loaded with 35 µg of lysate. Arrowheads indicate the detection of both mono-phosphorylated (upper) and unphosphorylated (lower) forms of the protein. After blocking with 5% NGS and 0.5% BLOTTO in PBS, the membrane was probed with the primary antibody diluted in blocking buffer to 1:600 for 2 h at room temperature. The membrane was washed and reacted with a 1:10,000 dilution of IRDye800™ conjugated Gt-a-Rabbit IgG [H&L] MX (611-132-122) for 45 min at room temperature (800 nm channel, green). Molecular weight estimation was made by comparison to prestained MW markers in lane M (700 nm channel, red). IRDye™800 fluorescence image was captured using the Odyssey® Infrared Imaging System developed by LI-COR. IRDye is a trademark of LI-COR, Inc. Other detection systems will yield similar results.

Application Note(s): This affinity-purified antibody was tested by ELISA and immunoblotting and was found to be reactive with both the unphosphorylated and mono-phosphorylated forms of the protein. Although not tested, this antibody is likely functional in immunohistochemistry and immunoprecipitation.

Recommended Dilution(s):

ELISA	1:5,000 - 1:20,000
WESTERN BLOT	1:500 - 1:2,000
IMMUNOHISTOCHEMISTRY	User Optimized
OTHER APPLICATIONS	User Optimized

Immunogen: This affinity purified antibody was prepared from whole rabbit serum produced by repeated immunizations with a synthetic peptide corresponding to amino acids 12-27 of human myosin light chain protein.

Relevant Links: NCBI P24844 (Human) NCBI Q9CQ19 (Mouse)

Purity and Specificity: This affinity purified antibody is directed against the regulatory light chain of smooth and non-muscle myosin. The antibody detects both unphosphorylated and monophosphorylated forms of the protein. The product was affinity purified from monospecific antiserum by immunoaffinity purification. Cross reactivity is expected with myosin light chain from human, mouse and rat sources. 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.

Protein Sequence: Human RLCM, 172 aa, predicted MW 19.8 kDa

1	msskrakakt	t**kkrpqrats**	**nvfamfd**qsq	iqefkeafnm	idqnrdgfid	kedlhdmlas
61	lgknptdeyl	egmmseapgp	inftmfltmf	geklngtdpe	dvirnafacf	deeasgfihe
121	dhlrellttm	gdrftdeevd	emyreapidk	kgnfnyveft	rilkhgakdk	dd

General Reference(s):

Kumar,C.C., Mohan,S.R., Zavodny,P.J., Narula,S.K. and Leibowitz,P.J. (1989) Characterization and differential expression of human vascular smooth muscle myosin light chain 2 isoform in nonmuscle cells. *Biochemistry* **28** (9), 4027-4035.

Iwasaki,T., Murata-Hori,M., Ishitobi,S. and Hosoya,H. (2001) Diphosphorylated MRLC is required for organization of stress fibers in interphase cells and the contractile ring in dividing cells. *Cell Struct. Funct*. **26** (6), 677-683.

Related Products:

#600-401-416		Affinity Purified Phosphospecific Anti-RLC of Smooth and Non-muscle Myosin at pS19/pS20 [Rabbit]
#600-401-938		Affinity Purified Anti-Regulatory Light Chain of Smooth and Non-muscle Myosin [Rabbit]
#611-703-127		Peroxidase Conjugated Affinity Purified Anti-RABBIT IgG (H&L) (DONKEY) MX10
#611-132-122		IRDye800 Conjugated Affinity Purified Anti-RABBIT IgG (H&L) (GOAT) MX10
#B501-0500	500 g	BLOTTO
#BSA-30	500 ml	30% BOVINE SERUM ALBUMIN SOL'N in 0.85% sodium chloride (no preservative or stabilizer)
#B304	10 ml	NORMAL GOAT SERUM (NGS)
#KIA-003		***Max*Tag**™ Anti-RABBIT IgG Kit for Immunoblotting
#MB-070		Blocking Buffer for Fluorescent Western Blotting

USDA Certification: All products of animal origin manufactured by Rockland Immunochemicals are derived from starting materials of North American origin. Collection was performed in United States Department of Agriculture (USDA) inspected facilities and all materials have been inspected and certified to be free of disease and suitable for exportation.

Note: This product is for research use only and is not intended for therapeutic or diagnostic applications. Please contact a technical service representative for more information.