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ProductInformation

Anti-phospho-Myosin Light Chain (pSer¹⁹) produced in rabbit, affinity isolated antibody

Catalog Number M6068

Synonym: Anti-MLC

Product Description

Anti-phospho-Myosin Light Chain (pSer¹⁹) is produced in rabbit using a synthetic phosphopeptide derived from the region of chicken MLC that contains serine 19 (serine 20 including the initiating methionine) as immunogen. The antiserum is affinity purified using epitope-specific affinity chromatography. The antibody is preadsorbed to remove any reactivity toward a non-phosphorylated peptide.

The antibody detects chicken MLC. Human (100% homologous) has not been tested, but is expected to react. It has been used in immunoblotting applications.

Myosin light chain is a subunit of the conventional myosins, e.g., myosin II. In smooth muscle and nonmuscle cells, conventional myosins mediate a wide variety of contractile events including cytokinesis, cell motility, and smooth muscle contraction. MLC is phosphorylated by multiple serine-threonine kinases such as Rho-kinase and PAK, however, myosin light chain kinase (MLCK) acts as the primary kinase. Contractile activity of conventional myosins is regulated by phosphorylation of MLC on several residues.

Phosphorylation of serine 19, the major phosphorylation site and the preferred site for MLCK, activates myosin motor activity under physiological conditions.

Reagent

Supplied in Dulbecco's phosphate buffered saline (without Mg²⁺ and Ca²⁺), pH 7.3, 50% glycerol, 1.0 mg/mL BSA (IgG and protease free) and 0.05% sodium azide.

Precautions and Disclaimer

This product is for R&D use only, not for drug, household, or other uses. Please consult the Material Safety Data Sheet for information regarding hazards and safe handling practices.

Storage/Stability

Store at -20 °C. Upon initial thawing, freeze the solution in working aliquots for extended storage. Avoid repeated freezing and thawing to prevent denaturing the antibody. Do not store in frost-free freezers. Working dilution samples should be discarded if not used within 12 hours. The antibody is stable for at least 12 months when stored appropriately.

Product Profile

The supplied reagent is sufficient for 10 blots.

Immunoblotting: a working dilution of 1:1,000 is recommended using chicken gizzard myosin II phosphorylated *in vitro* by PAK.

Note: Prior immunoprecipitation of MLC may be necessary.

Note: In order to obtain best results in various techniques and preparations, we recommend determining optimal working concentration by titration.

Peptide Competition

Results show that reactivity with the antibody requires phosphorylation of MLC by active kinase, and that only the peptide corresponding to MLC (pSer¹⁹) blocks the antibody signal, thereby demonstrating the specificity of the antibody.

References

- Chen, B.H., et al., Roles of Rho-associated kinase and myosin light chain kinase in morphological and migratory defects of focal adhesion kinase-null cells. *J. Biol. Chem.*, 277, 33857-33863 (2002).
- 2. Ueda, K., et al., Rho-kinase contributes to diphosphorylation of myosin II regulatory light chain in nonmuscle cells. *Oncogene*, **21**, 5852-5860 (2002).
- 3. Saitoh, T., et al., Differential localization of non-muscle myosin II isoforms and phosphorylated regulatory light chains in human MRC-5 fibroblasts. *FEBS Lett.*, **509**, 365-369 (2001).

- 4. Kolega, J., Turnover rates at regulatory phosphorylation sites on myosin II in endothelial cells. *J. Cell. Biochem.*, **75**, 629-639 (1999).
- Ikebe, R., et al., Role of the N-terminal region of the regulatory light chain in the dephosphorylation of myosin by myosin light chain phosphatase. *J. Biol. Chem.*, 274, 30122-30126 (1999).

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