

p-MYLK (Ser 439): sc-16800

BACKGROUND

The Ca²⁺/calmodulin-dependent protein kinase (CaM kinases) are a structurally related subfamily of serine/threonine kinases that includes CaMKI, CaMKII, CaMKIV and myosin light chain kinases (MLCKs). MLCK isoforms include non-muscle MLCK, smooth muscle MLCK and skeletal muscle MLCK (MLCKSK). Endothelial cell MLCK triggers actomyosin contractions essential for vascular barrier regulation and leukocyte diapedesis. Two high molecular weight MLCK splice variants in human endothelium, MLCK-1 and MLCK-2, are identical except for a deleted region in MLCK-2, which encodes a 69 amino acid stretch that contains potentially important consensus sites for phosphorylation by p60src kinase. The sites of tyrosine phosphorylation on MLCK-1 that are catalyzed by p60Src are Tyr 464 and Tyr 471, and they reside within the 69 residue stretch that is deleted in the MLCK-2 splice variant. Thus, p60Src-mediated tyrosine phosphorylation may provide an important mechanism for splice variant-specific regulation of non-muscle MLCK and vascular cell function.

REFERENCES

1. Roush, C.L., Kennelly, P.J., Glaccum, M.B., Helfman, D.M., Scott, J.D. and Krebs, E.G. 1988. Isolation of the cDNA encoding rat skeletal muscle myosin light chain kinase. Sequence and tissue distribution. *J. Biol. Chem.* 263: 10510-10516.
2. Kitani, T., Okuno, S. and Fujisawa, H. 1994. cDNA cloning and expression of human calmodulin-dependent protein kinase IV. *J. Biochem.* 115: 637-640.
3. Haribabu, B., Hook, S.S., Selbert, M.A., Goldstein, E.G., Tomhave, E.D., Edelman, A.M., Snyderman, R. and Means, A.R., 1995. Human calcium-calmodulin dependent protein kinase I: cDNA cloning, domain structure and activation by phosphorylation at Threonine 177 by calcium-calmodulin dependent protein kinase I kinase. *EMBO J.* 14: 3679-3686.

CHROMOSOMAL LOCATION

Genetic locus: MYLK (human) mapping to 3q21.

SOURCE

p-MYLK (Ser 439) is available as either goat (sc-16800) or rabbit (sc-16800-R) polyclonal affinity purified antibody raised against a short amino acid sequence containing phosphorylated Ser 439 of MYLK of human origin.

PRODUCT

Each vial contains 200 µg IgG in 1.0 ml of PBS with < 0.1% sodium azide and 0.1% gelatin.

Blocking peptide available for competition studies, sc-16800 P, (100 µg in 0.5 ml PBS containing < 0.1% sodium azide and 0.2% BSA).

STORAGE

Store at 4° C, ****DO NOT FREEZE****. Stable for one year from the date of shipment. Non-hazardous. No MSDS required.

APPLICATIONS

p-MYLK (Ser 439) is recommended for detection of Ser 439 phosphorylated MYLK of human origin by Western Blotting (starting dilution 1:200, dilution range 1:100-1:1000), immunoprecipitation [1-2 µg per 100-500 µg of total protein (1 ml of cell lysate)], immunofluorescence (starting dilution 1:50, dilution range 1:50-1:500) and solid phase ELISA (starting dilution 1:30, dilution range 1:30-1:3000).

Suitable for use as control antibody for MYLK siRNA (h): sc-35941, MYLK shRNA Plasmid (h): sc-35941-SH and MYLK shRNA (h) Lentiviral Particles: sc-35941-V.

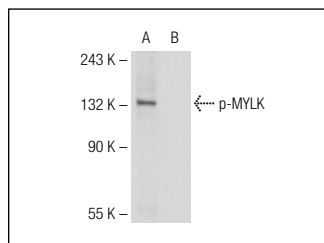
Molecular Weight of p-MYLK isoforms: 210/135 kDa.

Positive Controls: smooth muscle + Ca²⁺/calmodulin or MIA PaCa-2 cell lysate: sc-2285.

RECOMMENDED SECONDARY REAGENTS

To ensure optimal results, the following support (secondary) reagents are recommended: 1) Western Blotting: for goat primary antibody (sc-14268): use donkey anti-goat IgG-HRP: sc-2020 (range: 1:2000-1:100,000) or Cruz Marker™ compatible donkey anti-goat IgG-HRP: sc-2033 (range: 1:2000-1:5000), for rabbit primary antibody (sc-14268-R): use goat anti-rabbit IgG-HRP: sc-2004 (range: 1:2000-1:100,000) or Cruz Marker™ compatible goat anti-rabbit IgG-HRP: sc-2030 (range: 1:2000-1:5000); Cruz Marker™ Molecular Weight Standards: sc-2035, TBS Blotto B Blocking Reagent: sc-2335 (use 50 mM NaF, sc-24988, as diluent), Western Blotting Luminol Reagent: sc-2048 and Lambda Phosphatase: sc-200312A. 2) Immunoprecipitation: use Protein A/G PLUS-Agarose: sc-2003 (0.5 ml agarose/2.0 ml). 3) Immunofluorescence: for goat primary antibody (sc-14268): use donkey anti-goat IgG-FITC: sc-2024 (range: 1:100-1:400) or donkey anti-goat IgG-TR: sc-2783 (range: 1:100-1:400), for rabbit primary antibody (sc-14268-R): use goat anti-rabbit IgG-FITC: sc-2012 (range: 1:100-1:400) or goat anti-rabbit IgG-TR: sc-2780 (range: 1:100-1:400) with UltraCruz™ Mounting Medium: sc-24941.

DATA



p-MYLK (Ser 439)-R: sc-16800-R. Western blot analysis of MYLK phosphorylation in untreated (A) and lambda protein phosphatase (sc-200312A) treated (B) MIA PaCa-2 whole cell lysates.

RESEARCH USE

For research use only, not for use in diagnostic procedures.