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Specialists in Signaling Proteins
1-866-9 KINASE (54-6273)

Catalogue #	Aliquot Size
M63-10H-05	5 µg
M63-10H-10	10 µg
M63-10H-20	20 µg

MYLK2, Active

Full-length recombinant protein expressed in Sf9 cells

Catalog # M63-10H
Lot # E165-4

Product Description

Recombinant full-length human MYLK2 was expressed by baculovirus in Sf9 insect cells using an N-terminal His tag. The gene accession number is NM_033118.

Gene Aliases

skMLCK, KMLC, MLCK, MLCK2

Formulation

Recombinant protein stored in 50mM sodium phosphate, pH 7.0, 300mM NaCl, 150mM imidazole, 0.1mM PMSF, 0.25mM DTT, 25% glycerol.

Storage and Stability

Store product at –70°C. For optimal storage, aliquot target into smaller quantities after centrifugation and store at recommended temperature. For most favorable performance, avoid repeated handling and multiple freeze/thaw cycles.

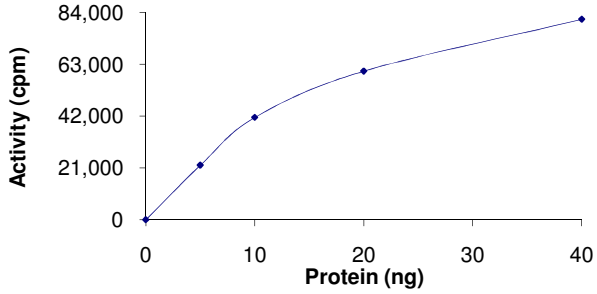
Scientific Background

MYLK2 is a member of the myosin light chain kinase family and is a calcium/calmodulin dependent enzyme that is exclusively expressed in adult skeletal muscle (1). MYLK2 has been proposed to participate in signaling pathways (calcium signaling pathway, focal adhesion, regulation of actin cytoskeleton) and cellular processes (neuromuscular synaptic transmission, protein/amino acid phosphorylation). MYLK2 is involved in multiple molecular functions as a result of various subdomains that participate in ATP binding, calmodulin binding, nucleotide binding, protein serine/threonine kinase activity and transferase activity) (2).

References

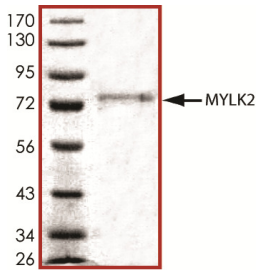
1. Soung, Y.H. et al:, Mutational analysis of the kinase domain of MYLK2 gene in common human cancers.Pathol Res Pract. 2006;202(3):137-40.
2. Toth-Zsamboki, E. et al. P2X1-mediated ERK2 activation amplifies the collagen-induced platelet secretion by enhancing myosin light chain kinase activation.J. Biol Chem. 2003; 21;278(47):46661-7.

Specific Activity



The specific activity of MYLK2 was determined to be **238 nmol/min/mg** as per activity assay protocol.

Purity



The purity of MYLK2 was determined to be **>85%** by densitometry.
Approx. MW **74kDa**.

MYLK2, Active

Full-length recombinant human protein expressed in Sf9 cells

Catalog Number	M63-10H
Specific Activity	238 nmol/min/mg
Specific Lot Number	E165-4
Purity	>85%
Concentration	0.1 µg/µl
Stability	1yr at –70°C from date of shipment
Storage & Shipping	Store product at –70°C. For optimal storage, aliquot target into smaller quantities after centrifugation and store at recommended temperature. For most favorable performance, avoid repeated handling and multiple freeze/thaw cycles. Product shipped on dry ice.

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Activity Assay Protocol

Reaction Components

Active Kinase (Catalog #: M63-10H)

Active MYLK2 (0.1μg/μl) diluted with Kinase Dilution Buffer III (Catalog #: K23-09) and assayed as outlined in sample activity plot. (Note: these are suggested working dilutions and it is recommended that the researcher perform a serial dilution of Active MLCK for optimal results).

Kinase Dilution Buffer III (Catalog #: K23-09)

Kinase Assay Buffer I (Catalog #: K01-09) diluted at a 1:4 ratio (5X dilution) with 50ng/μl BSA solution.

Kinase Assay Buffer I (Catalog #: K01-09)

Buffer components: 25mM MOPS, pH 7. 2, 12.5mM β-glycerol-phosphate, 25mM $MgC1_2$, 5mM EGTA, 2mM EDTA. Add 0.25mM DTT to Kinase Assay Buffer prior to use.

[^{33}P]-ATP Assay Cocktail

Prepare 250μM [^{33}P]-ATP Assay Cocktail in a designated radioactive working area by adding the following components: 150μl of 10mM ATP Stock Solution (Catalog #: A50-09), 100μl [^{33}P]-ATP (1mCi/100μl), 5.75ml of Kinase Assay Buffer I (Catalog #: K01-09). Store 1ml aliquots at –20°C.

10mM ATP Stock Solution (Catalog #: A50-09)

Prepare ATP stock solution by dissolving 55mg of ATP in 10ml of Kinase Assay Buffer I (Catalog #: K01-09). Store 200μl aliquots at –20°C.

Substrate (Catalog #: M89-54G)

LC20 protein substrate, 1mg/ml concentration.

Assay Protocol

Step 1. Thaw [^{33}P]-ATP Assay Cocktail in shielded container in a designated radioactive working area.

Step 2. Thaw the Active MYLK2, Kinase Assay Buffer, Substrate and Kinase Dilution Buffer on ice.

Step 3. In a pre-cooled microfuge tube, add the following reaction components bringing the initial reaction volume up to 20μl:

- **Component 1.** 10μl of diluted Active MYLK2 (Catalog #M63-10H)
- **Component 2.** 5μl of 1mg/ml stock solution of substrate (Catalog #M89-54G)
- **Component 3.** 2.5μl of Ca^{2+} / Calmodulin Solution II, 10x (Catalog #C02-39B)
- **Component 4.** 2.5μl of distilled H_2O

Step 4. Set up the blank control as outlined in step 3, excluding the addition of the substrate. Replace the substrate with an equal volume of distilled H_2O.

Step 5. Initiate the reaction by the addition of 5 μl [^{33}P]-ATP Assay Cocktail bringing the final volume up to 25μl and incubate the mixture in a water bath at 30°C for 15 minutes.

Step 6. After the 15 minute incubation period, terminate the reaction by spotting 20 μl of the reaction mixture onto individual pre-cut strips of phosphocellulose P81 paper.

Step 7. Air dry the pre-cut P81 strip and sequentially wash in a 1% phosphoric acid solution (dilute 10ml of phosphoric acid and make a 1L solution with distilled H_2O) with constant gentle stirring. It is recommended that the strips be washed a total of 3 intervals for approximately 10 minutes each.

Step 8. Count the radioactivity on the P81 paper in the presence of scintillation fluid in a scintillation counter.

Step 9. Determine the corrected cpm by removing the blank control value (see Step 4) for each sample and calculate the kinase specific activity as outlined below.

Calculation of [P^{33}]-ATP Specific Activity (SA) (cpm/pmol)

Specific activity (SA) = cpm for 5 μl [^{33}P]-ATP / pmoles of ATP (in 5 μl of a 250 μM ATP stock solution, i.e., 1250 pmoles)

Kinase Specific Activity (SA) (pmol/min/μg or nmol/min/mg)

Corrected cpm from reaction / [(SA of ^{33}P-ATP in cpm/pmol)*(Reaction time in min)*(Enzyme amount in μg or mg)]*[(Reaction Volume) / (Spot Volume)]

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