Store at -80°C

#7498

MYLK2 Kinase

☑5 µg

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New 11/06

Description: Purified recombinant full length human MYLK2 (Met1-Val596) kinase, supplied as a GST fusion protein.

Background: Myosin light chain kinases (MYLKs) are Ca^{2+}/calmodulin-dependent protein kinases that regulate myosin motor activities through phosphorylation of myosin regulatory light chain (RLC). This phosphorylation increases the actin-activated myosin ATPase activity and is thought to play major roles in a number of biological processes, including cell spreading and migration, transepithelial permeability and muscle contraction (1). In vertebrates there are 2 genes for MYLK. The skeletal MYLK (MYLK2) is expressed predominantly in skeletal muscles and regulates tension potentiation due to repetitive stimulation in fast-twitch skeletal muscles (2). The smooth muscle MYLK is more ubiquitously expressed with the greatest expression observed in smooth muscle tissue (3). Skeletal MYLK can phosphorylate myosin light chains isolated from skeletal or smooth muscle. In contrast, smooth muscle MYLK specifically phosphorylates myosin light chains isolated from smooth muscle (4).

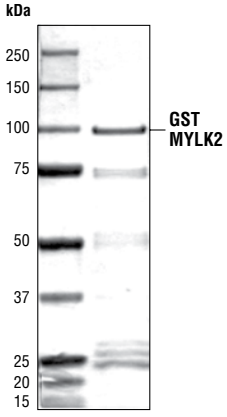

Figure 1. The purity of the GST-MYLK2 fusion protein was analyzed using SDS/PAGE followed by Coomassie stain.

Source/Purification: The GST-Kinase fusion protein was produced using a baculovirus expression system with a construct expressing full length human MYLK2 (Met1-Val596) (GenBank Accession No. NM_033118.2) with an amino-terminal GST tag. The protein was purified by one-step affinity chromatography using GSH-agarose.

Quality Control: The theoretical molecular weight of the GST-MYLK2 fusion protein is 95 kDa. The purified kinase was quality controlled for purity using SDS-PAGE followed by Coomassie stain [Fig.1]. MYLK2 kinase activity was determined using a radiometric assay [Fig.2].

Background References:

(1) Kamm, K.E. and Stull, J.T. (2001) *J. Biol. Chem.* 276, 4527-4530.

(2) Zhi, G. et al. (2005) *Proc. Natl. Acad. Sci. USA* 102, 17519–17524.

(3) Lazar, V. and Garcia, J.G. (1999) *Genomics* 57, 256–267.

(4) Herring, B.P. et al. (1992) *J. Biol. Chem.* 267, 25945–25950.

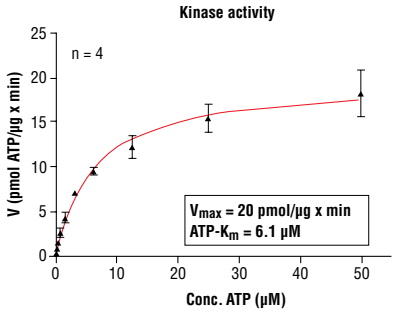

Figure 2. MYLK2 kinase activity was measured in a radiometric assay using the following reaction conditions: 60 mM HEPES-NaOH, pH 7.5, 3 mM $MgCl_2$, 3 mM $MnCl_2$, 0.4 mM $CaCl_2$, 3 µM Na-orthovanadate, 1.2 mM DTT, 0.04 mg/ml calmodulin, 2.5 µg/50 µl PEG20,000, ATP variable, Substrate: GSK3 derived peptide (R11-SGRARTSSFAEPGGK) 200 ng/µL, and recombinant MYLK2: 200 ng/50 µl.

Storage: Enzyme is supplied in 50 mM Tris-HCl, pH 7.5; 150 mM NaCl, 0.25 mM DTT, 0.1 mM EGTA, 0.1 mM EDTA, 0.1 mM PMSF, 25% glycerol, 7 mM glutathione. Store at –80°C.

Keep on ice during use.

Avoid repeated freeze-thaw cycles.

Companion Products:

Serine/Threonine Kinase Substrate Screening Kit #7400

Kinase Buffer (10X) #9802

ATP (10 mM) #9804

Protocol for MYLK2 Kinase Assay

Note: Lot-specific information for this kinase is provided on the enzyme vial. Optimal assay incubation times and enzyme concentrations must be determined empirically for each lot of kinase under specified conditions.

A Additional Solutions and Reagents (Not included)

1. **Kinase Buffer (5X)**
   300 mM HEPES-NaOH, pH 7.5
   15 mM $MgCl_2$
   15 mM $MnCl_2$
   15 µM Na-orthovanadate
   6 mM DTT
   12.5 µg/50 µl $PEG_{20,000}$
2. ATP (10 mM) #9804
3. ^{32}P-γATP
4. 0.5 mg/ml calmodulin in 5 mM $CaCl_2$
5. GSK3 derived peptide (R11-SGRARTSSFAEPGGK) (500 ng/µL)

B Suggested Protocol

1. Dilute 10 mM ATP with 3X assay buffer 1:40 to make 250 µM ATP.
2. Dilute [^{32}p] ATP to 0.16 µCi/µl [^{32}p] ATP with 250 µM ATP solution.
3. Transfer enzyme from -80°C to ice. Allow enzyme to thaw on ice.
4. Dilute MYLK2 kinase protein to 20 ng/µl with 1X assay buffer followed by 2-fold serial dilutions.
5. To start the reaction combine 10 µl diluted MYLK2 kinase solution, 10 µl GSK3 derived peptide (500 ng/µL), 2 µL 0.5 mg/ml calmodulin in 5 mM $CaCl_2$, and 5 µl 0.16 µCi/µl [^{32}p] ATP solution.

Final Assay Conditions
60 mM HEPES-NaOH, pH 7.5
3 mM $MgCl_2$
3 mM $MnCl_2$
0.4 mM $CaCl_2$
3 µM Na-orthovanadate
1.2 mM DTT
50 ng/µl $PEG_{20,000}$
0.04 mg/ml calmodulin
200 ng/µL GSK3 derived peptide

6. After 15 minutes terminate reaction by spotting 20 µl of the reaction mixture onto phosphocellulose P81 paper.
7. Air dry the P81 paper then wash with 1% phosphoric acid 3 times.
8. Transfer P81 paper to 4 ml scintillation tube then add 3 ml scintillation cocktail.
9. Count samples in a scintillation counter.

Cell Signaling Technology offers a full line of protein kinases, substrates, and antibody detection reagents for high throughput screening. Please direct all inquiries to: drugdiscovery@cellsignal.com.