3050 Spruce Street, St. Louis, MO 63103 USA
Tel: (800) 521-8956 (314) 771-5765 Fax: (800) 325-5052 (314) 771-5757
email: techservice@sial.com sigma-aldrich.com

Product Information

MYLK2, active, His-tagged, human
PRECISIO® Kinase
recombinant, expressed in *Sf*9 cells

Catalog Number **M0949**
Lot Number 041M0980
Storage Temperature –70 °C

Synonyms: skMLCK, KMLC, MLCK, MLCK2

Product Description

MYLK2 is a member of the myosin light chain kinase family and is a calcium/calmodulin dependent enzyme exclusively expressed in adult skeletal muscle.[1] MYLK2 has been proposed to participate in signaling pathways (calcium signaling pathway, focal adhesion, and regulation of actin cytoskeleton) and cellular processes (neuromuscular synaptic transmission and 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]

This recombinant product was expressed by baculovirus in *Sf*9 insect cells using an N-terminal His-tag. The gene accession number is NM 033118. It is supplied in 50 mM sodium phosphate, pH 7.0, with 300 mM NaCl, 150 mM imidazole, 0.2 mM DTT, 0.1 mM PMSF, and 25% glycerol.

Molecular mass: ~74 kDa

Purity: ≥70% (SDS-PAGE, see Figure 1)

Specific Activity: 229–311 nmole/min/mg (see Figure 2)

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

The product ships on dry ice and storage at –70 °C is recommended. After opening, aliquot into smaller quantities and store at –70 °C. Avoid repeated handling and multiple freeze/thaw cycles.

Figure 1.
SDS-PAGE Gel of Lot Number 041M0980:
>75% (densitometry)

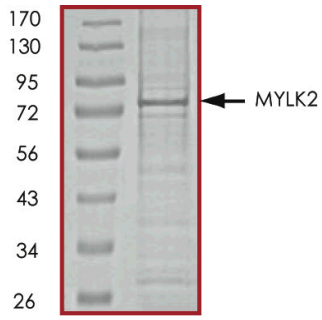
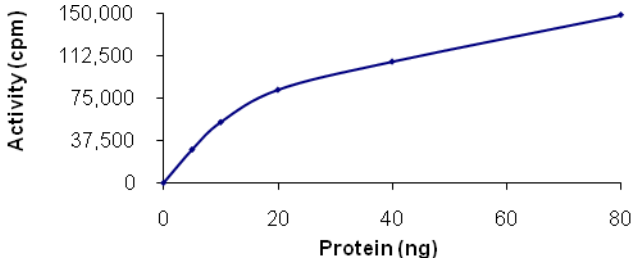


Figure 2.
Specific Activity of Lot Number 041M0980:
270 nmole/min/mg



Procedure

Preparation Instructions

Kinase Assay Buffer – 25 mM MOPS, pH 7.2, 12.5 mM glycerol 2-phosphate, 25 mM $MgCl_2$, 5 mM EGTA, and 2 mM EDTA. Just prior to use, add DTT to a final concentration of 0.25 mM.

Kinase Dilution Buffer – Dilute the Kinase Assay Buffer 5-fold with a 50 ng/µl BSA solution.

Kinase Solution – Dilute the active MYLK2 (0.1 µg/µl) with Kinase Dilution Buffer to the desired concentration. Note: The lot-specific specific activity plot may be used as a guideline (see Figure 2). It is recommended the researcher perform a serial dilution of active MYLK2 kinase for optimal results.

10 mM ATP Stock Solution – Dissolve 55 mg of ATP in 10 ml of Kinase Assay Buffer. Store in 200 µl aliquots at –20 °C.

γ-^{32}P-ATP Assay Cocktail (250 µM) – Combine 5.75 ml of Kinase Assay Buffer, 150 µl of 10 mM ATP Stock Solution, 100 µl of γ-^{32}P-ATP (1 mCi/100 µl). Store in 1 ml aliquots at –20 °C.

Substrate Solution – Prepare LC20 protein substrate at a final concentration of 1.0 mg/ml.

1% phosphoric acid solution – Dilute 10 ml of concentrated phosphoric acid to a final volume of 1 L with water.

Kinase Assay

This assay involves the use of the ^{32}P radioisotope. All institutional guidelines regarding the use of radioisotopes should be followed.

1. Thaw the active MYLK2, Kinase Assay Buffer, Substrate Solution, and Kinase Dilution Buffer on ice. The γ-^{32}P-ATP Assay Cocktail may be thawed at room temperature.
2. In a pre-cooled microcentrifuge tube, add the following solutions to a volume of 20 µl:
   - 10 µl of Kinase Solution
   - 5 µl of Substrate Solution
   - 2.5 µl of 5 mM $CaCl_2$ solution containing 0.75 µg Calmodulin
   - 2.5 µl of water
3. Set up a blank control as outlined in step 2, substituting 5 µl of cold water (4 °C) for the Substrate Solution.
4. Initiate each reaction with the addition of 5 µl of the γ-^{32}P-ATP Assay Cocktail, bringing the final reaction volume to 25 µl. Incubate the mixture in a water bath at 30 °C for 15 minutes.
5. After the 15 minute incubation, stop the reaction by spotting 20 µl of the reaction mixture onto an individually precut strip of phosphocellulose P81 paper.
6. Air dry the precut P81 strip and sequentially wash in the 1% phosphoric acid solution with constant gentle stirring. It is recommended the strips be washed a total of 3 times of ~10 minutes each.
7. Set up a radioactive control to measure the total γ-^{32}P-ATP counts introduced into the reaction. Spot 5 µl of the γ-^{32}P-ATP Assay Cocktail on a precut P81 strip. Dry the sample for 2 minutes and read the counts. Do not wash this sample.
8. Count the radioactivity on the P81 paper in the presence of scintillation fluid in a scintillation counter.
9. Determine the corrected cpm by subtracting the blank control value (see step 3) from each sample and calculate the kinase specific activity

Calculations:

1. Specific Radioactivity (SR) of ATP (cpm/nmole)

$$SR = \frac{\text{cpm of 5 µl of } \gamma\text{-}^{32}\text{P-ATP Assay Cocktail}}{\text{nmole of ATP}}$$

   cpm – value from control (step 7)
   nmole – 1.25 nmole (5 µl of 250 µM ATP Assay Cocktail)

2. Specific Kinase Activity (SA) (nmole/min/mg)

$$\text{nmole/min/mg} = \frac{\Delta\text{cpm} \times (25/20)}{SR \times E \times T}$$

SR = specific radioactivity of the ATP (cpm/nmole ATP)
Δcpm = cpm of the sample – cpm of the blank (step 3)
25 = total reaction volume
20 = spot volume
T = reaction time (minutes)
E = amount of enzyme (mg)

References

1. Soung, Y.H. et al., Mutational analysis of the kinase domain of MYLK2 gene in common human cancers. Pathol. Res. Pract., **202**, 137-140 (2006).
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., **278**, 46661-46667 (2003).

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