

Catalogue #	Aliquot Size
<b>M64-10G-05</b>	<b>5 µg</b>
<b>M64-10G-10</b>	<b>10 µg</b>
<b>M64-10G-20</b>	<b>20 µg</b>

## MYLK3, Active

Full-length recombinant protein expressed in Sf9 cells

**Catalog # M64-10G**

Lot # E117-1

### Product Description

Recombinant full-length human MYLK3 was expressed by baculovirus in Sf9 insect cells using an N-terminal GST tag. The gene accession number is [BC109097](#).

### Gene Aliases

caMLCK; MLCK, MLCK2, MGC126319, MGC126320

### Formulation

Recombinant protein stored in 50mM Tris-HCl, pH 7.5, 150mM NaCl, 10mM glutathione, 0.1mM EDTA, 0.25mM DTT, 0.1mM PMSF, 25% glycerol

### Storage and Stability

Store product at  $-70^{\circ}\text{C}$ . 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.

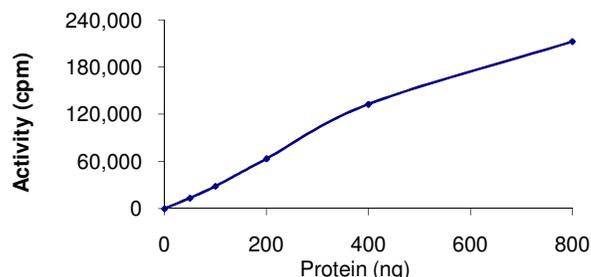
### Scientific Background

MYLK3 (or myosin light chain kinase 3) plays a key role in rate of cross-bridge recruitment in cardiac myocytes. MYLK3 is upregulated in failing human myocardia and expression of MYLK3 correlates with the pulmonary arterial pressure of patients with heart failure (1). Knockdown of MYLK3 with small interfering RNAs in cultured rat cardiomyocytes decreases Myl2 phosphorylation and impairs epinephrine-induced activation of sarcomere reassembly (2). Recombinant murine MYLK3 shows specificity for Myl2 (Mlc2v) and the phosphorylation is calcium- and calmodulin-dependent.

### References

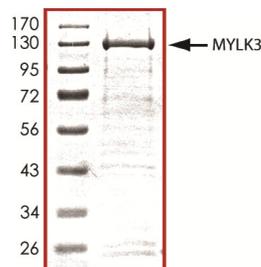
- Chan, J. Y. et al: Identification of cardiac-specific myosin light chain kinase. *Circ. Res.* 2008; 102: 571-580.
- Seguchi, O. et al: A cardiac myosin light chain kinase regulates sarcomere assembly in the vertebrate heart. *J. Clin. Invest.* 2007; 117: 2812-2824.

### Specific Activity



The specific activity of MYLK3 was determined to be **11 nmol/min/mg** as per activity assay protocol.

### Purity



The purity of MYLK3 was determined to be **>85%** by densitometry. Approx. MW **135kDa**.

## MYLK3, Active

Full-length recombinant human protein expressed in Sf9 cells

Catalog Number	M64-10G
Specific Activity	11 nmol/min/mg
Specific Lot Number	E117-1
Purity	>85%
Concentration	0.1 µg/µl
Stability	1yr at $-70^{\circ}\text{C}$ from date of shipment
Storage & Shipping	Store product at $-70^{\circ}\text{C}$ . 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 #: M64-10G)

Active MYLK3 (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 MYLK3 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 MgCl<sub>2</sub>, 5mM EGTA, 2mM EDTA. Add 0.25mM DTT to Kinase Assay Buffer prior to use.

### [<sup>33</sup>P]-ATP Assay Cocktail

Prepare 250µM [<sup>33</sup>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 [<sup>33</sup>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, 0.2mg/ml concentration.

## Assay Protocol

- Step 1.** Thaw [<sup>33</sup>P]-ATP Assay Cocktail in shielded container in a designated radioactive working area.
- Step 2.** Thaw the Active MYLK3, 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 MYLK3 (Catalog #M64-10G)
  - Component 2.** 7.5µl of 1mg/ml stock solution of substrate (Catalog #M89-54G)
  - Component 3.** 2.5µl of Ca<sup>2+</sup> / Calmodulin Solution II, 10x (Catalog #C02-39B)
- 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<sub>2</sub>O.
- Step 5.** Initiate the reaction by the addition of 5 µl [<sup>33</sup>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<sub>2</sub>O) 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 [<sup>33</sup>P]-ATP Specific Activity (SA) (cpm/pmol)

Specific activity (SA) = cpm for 5 µl [<sup>33</sup>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 <sup>33</sup>P-ATP in cpm/pmol)\*(Reaction time in min)\*(Enzyme amount in µg or mg)]\*[(Reaction Volume) / (Spot Volume)]

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