

## MLCK, Active

Recombinant human protein expressed in Sf9 cells

Catalog # M62-11G-10

Lot # P012-2

### Product Description

Recombinant human MLCK (1425-1776) was expressed by baculovirus in Sf9 insect cells using an N-terminal GST tag. The gene accession number is [NM\\_053025](#).

### Gene Aliases

MYLK, KRP, MLCK108, MLCK210, MSTP083, FLJ12216

### Concentration

0.1 µg/µl

### 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, Shipping 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. Stability is 1yr at -70°C from date of shipment. Product shipped on dry ice.

### Scientific Background

MLCK or myosin light chain kinase is a muscle member of the immunoglobulin gene superfamily and is a calcium/calmodulin dependent protein kinase. MLCK is a regulatory protein for smooth muscle contraction, which acts by phosphorylating 20-kDa myosin light chain (MLC20) to activate the myosin ATPase activity. The kinase activity for the phosphorylation is localized at the central part of MLCK, which is also furnished with actin-binding activity at its N terminal and myosin-binding activity at its C terminal (1). Phosphorylation of regulatory light chain of myosin plays an important role in controlling the morphological changes seen during cell division (2).

### References

- Gao, Y. et al: Myosin light chain kinase as a multifunctional regulatory protein of smooth muscle contraction. IUBMB Life. 2001 Jun;51(6):337-44.
- Matsumura, F. et al: Role of myosin light chain phosphorylation in the regulation of cytokinesis. Cell Struct Funct. 2001 Dec;26(6):639-44.

### Purity

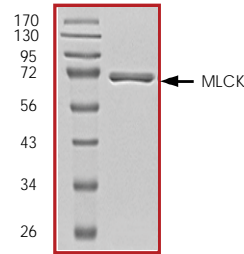
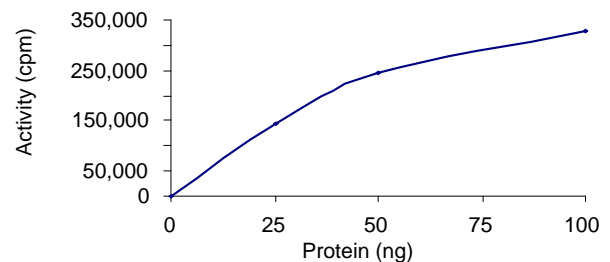


Figure 1. SDS-PAGE gel image

The purity of MLCK was determined to be **>95%** by densitometry, approx. MW **70kDa**.

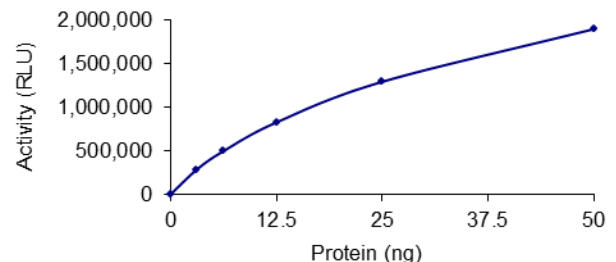
### Specific Activity

Figure 2. Radiometric Assay Data



The specific activity of MLCK was determined to be **200 nmol /min/mg** as per activity assay protocol.  
(For Radiometric Assay Protocol on this product please see pg. 2)

Figure 3. ADP- Glo™ Assay Data



The specific activity of MLCK was determined to be **850 nmol /min/mg** as per activity assay protocol.  
(For ADP-Glo™ Assay Protocol on this product please see pg. 3)

# Activity Assay Protocol

## Reaction Components

### Active Kinase (Catalog #: M62-11G)

Active MLCK (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 50 ng/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 #: M56-59)

MRCL3 synthetic peptide substrate (KKRPQRATSN-VFAM-NH<sub>2</sub>) diluted in distilled H<sub>2</sub>O to a final concentration of 1mg/ml.

## 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 MLCK, 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 MLCK (Catalog #M62-11G)
  - Component 2. 5µl of 1mg/ml stock solution of substrate (Catalog #M56-59)
  - Component 3. 2.5µl of Ca<sup>2+</sup>/Calmodulin solution (10X) (Catalog #C02-39)
  - Component 4. 2.5µl distilled H<sub>2</sub>O (4°C)
- 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 5µl [<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 20µl 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 (cpm) 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 5µl [<sup>33</sup>P]-ATP / pmoles of ATP (in 5µl of a 250µM ATP stock solution, i.e., 1,250 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)]

# ADP-Glo™ Activity Assay Protocol

## Reaction Components

### MLCK Kinase Enzyme System (Promega, Catalog #:V4496)

MLCK, Active, 10µg (0.1µg/µl)  
MRCL3 Peptide, 1ml (1mg/ml)  
Reaction Buffer A (5X), 1.5ml  
DTT solution (0.1M), 25µl  
Ca<sup>2+</sup>/Calmodulin solution (10X), 500µl

### ADP-Glo™ Kinase Assay Kit (Promega, Catalog #: V9101)

Ultra Pure ATP solution, 10 mM (0.5ml)  
ADP solution, 10 mM (0.5ml)  
ADP-Glo™ Reagent (5ml)  
Kinase Detection Buffer (10ml)  
Kinase Detection Substrate (Lyophilized)

### Reaction Buffer A (5X)

200mM Tris-HCl, pH 7.5, 100mM MgCl<sub>2</sub> and 0.5 mg/ml BSA.

## Assay Protocol

The MLCK assay is performed using the MLCK Kinase Enzyme System (Promega; Catalog #: V4496) and ADP-Glo™ Kinase Assay kit (Promega; Catalog #: V9101). The MLCK reaction utilizes ATP and generates ADP. Then the ADP-Glo™ Reagent is added to simultaneously terminate the kinase reaction and deplete the remaining ATP. Finally, the Kinase Detection Reagent is added to convert ADP to ATP and the newly synthesized ATP is converted to light using the luciferase/luciferin reaction. For more detailed protocol regarding *the ADP-Glo™ Kinase Assay*, see the Technical Manual #TM313, available at [www.promega.com/tbs/tm313/tm313.html](http://www.promega.com/tbs/tm313/tm313.html).

- Step 1.** Thaw the ADP-Glo™ Reagents at ambient temperature. Then prepare Kinase Detection Reagent by mixing Kinase Detection Buffer with the Lyophilized Kinase Detection Substrate. Set aside.
- Step 2.** Thaw the components of MLCK Enzyme System, ADP and ATP on ice.
- Step 3.** Prepare 1ml of 2X Buffer by combining 400µl Reaction Buffer A, 1µl DTT and 599µl of dH<sub>2</sub>O.
- Step 4.** Prepare 1ml of 250µM ATP Assay Solution by adding 25µl ATP solution (10mM) to 500µl of 2X Buffer and 475µl of dH<sub>2</sub>O.
- Step 5.** Prepare diluted MLCK in 1X Buffer (diluted from 2X buffer) 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).
- Step 6.** In a white 96-well plate (Corning Cat # 3912), add the following reaction components bringing the initial reaction volume up to 20µl:

|                     |  |
|---------------------|--|
| <b>Component 1.</b> | 5µl of diluted Active MLCK                           |
| <b>Component 2.</b> | 5µl of 1mg/ml stock solution of substrate            |
| <b>Component 3.</b> | 2.5µl of Ca <sup>2+</sup> /Calmodulin solution (10X) |
| <b>Component 4.</b> | 7.5µl of 2X Buffer                                   |

- Step 7.** Set up the blank control as outlined in step 6, excluding the addition of the substrate. Replace the substrate with an equal volume of distilled H<sub>2</sub>O.
- Step 8.** At the same time as the MLCK kinase reaction, set up an ATP to ADP conversion curve at 50µM ATP/ADP range as described in the *ADP-Glo™ Kinase Assay* Technical Manual #TM313.
- Step 9.** Initiate the MLCK reactions by the addition of 5µl of 250µM ATP Assay Solution thereby bringing the final volume up to 25µl. Shake the plate and incubate the reaction mixture at 30°C for 15 minutes.
- Step 10.** Terminate the reaction and deplete the remaining ATP by adding 25µl of ADP-Glo™ Reagent. Shake the 96-well plate and then incubate the reaction mixture for another 40 minute at ambient temperature.
- Step 11.** Add 50µl of the Kinase Detection Reagent, shake the plate and then incubate the reaction mixture for another 30 minute at ambient temperature.
- Step 12.** Read the 96-well reaction plate using the Kinase-Glo™ Luminescence Protocol on a GloMax® plate reader (Promega; Cat# E7031).
- Step 13.** Using the conversion curve, determine the amount of ADP produced (nmol) in the presence (step 6) and absence of substrate (Step 7) and calculate the kinase specific activity as outlined below. For a detailed protocol of how to determine nmols from RLUs, see ADP-Glo™ Applications Database at <http://www.promega.com/applications/cellularanalysis/cellsignaling.htm>

### Kinase Specific Activity (SA) (nmol/min/mg)

(ADP (step 6) – ADP (Step 7)) in nmol / (Reaction time in min)\*(Enzyme amount in mg)