Store at –80°C

#7352

MARK1 Kinase

☑5 µg

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New 02/07

Description: Purified recombinant full length-human MARK1 kinase, supplied as a GST fusion protein.

Background: Microtubule associated proteins regulate the stability of microtubules, controlling processes such as cell polarity/differentiation, neurite outgrowth, cell division and organelle trafficking (1). The MARK (MAP/microtubule affinity-regulating kinases) family (MARK1-4) of serine/threonine kinases were identified based on their ability to phosphorylate microtubule associated proteins (MAPs) including tau, MAP2 and MAP4 (2-6). MARKs phosphorylate MAPs within their microtubule binding domains, causing dissociation of MAPs from microtubules and increased microtubule dynamics (2-4). In the case of tau, phosphorylation has been hypothesized to contribute to the formation of neurofibrillary tangles observed in Alzheimer's disease. Overexpression of MARK leads to hyperphosphorylation of MAPs, morphological changes and cell death (4). The tumor suppressor kinase LKB1 phosphorylates MARK and the closely related AMP-kinases within their T-loops, leading to increased activity (7).

Source/Purification: The GST-Kinase fusion protein was produced using a baculovirus expression system using sf9 cells and a recombinant virus encoding for full-length human MARK1 (Met1- Leu780) (GenBank Accession No. NM_018650) with an amino-terminal GST tag. The protein was purified by one-step affinity chromatography using GSH-agarose.

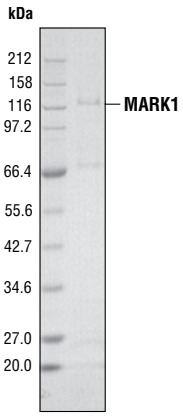

Figure 1. The purity of the GST-MARK1 fusion protein was analyzed using SDS/PAGE followed by Coomassie stain.

Quality Control: The theoretical molecular weight of the GST-MARK1 fusion protein is 117 kDa. The purity of the kinase was assessed using SDS-PAGE followed by Coomassie stain [Fig.1]. MARK1 kinase activity was determined using a radiometric assay [Fig.2].

Background References:

(1) Drubin, D.G. and Nelson, W.J. (1996) *Cell* 84, 335–344.

(2) Illenberger, S. et al. (1996) *J. Biol. Chem.* 271, 10834–10843.

(3) Drewes, G. et al. (1995) *J. Biol. Chem.* 270, 7679–7688.

(4) Drewes, G. et al. (1997) *Cell* 89, 297–308.

(5) Kato, T. et al. (2001) *Neoplasia* 3, 4–9.

(6) Trinczek, B. et al. (2004) *J. Biol. Chem.* 279, 5915–5923.

(7) Lizcano, J.M. et al. (2004) *EMBO J.* 23, 833–843.

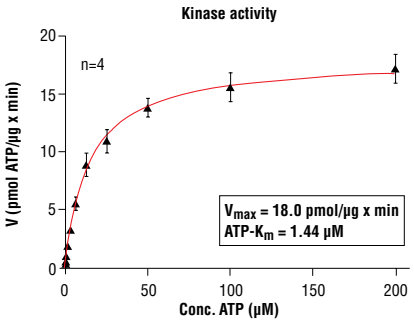

Figure 2. MARK1 kinase activity was measured in a radiometric assay using the following reaction conditions: 60 mM HEPES-NaOH, pH 7.5, 3 mM $MgCl_2$, 3 mM $MnCl_2$, 3 µM Na-orthovanadate, 1.2 mM DTT, 50 µg/µl PEG20,000, ATP variable, Substrate: CHKtide 5 ng/µL and recombinant MARK1: 200 ng/ 50 µl.

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

#7352

Protocol for MARK1 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
   250 µg/µl $PEG_{20,000}$
2. ATP (10 mM) #9804
3. ^{32}P-γATP
4. CHKtide (12.5 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 MARK1 protein to 20 ng/µl with 1X assay buffer followed by 2-fold serial dilutions.
5. To start the reaction combine 10 µl diluted MARK1 kinase solution, 10 µl CHKtide (12.5 ng/µl), and 5 µl 0.16 µCi/µl [^{32}p] ATP solution.

Final Assay Conditions
60 mM HEPES-NaOH, pH 7.5
3 mM $MgCl_2$
3mM $MnCl_2$
3 µM Na-orthovandate
1.2 mM DTT
40 ng/Ml $PEG_{20,000}$
5 ng/µl CHKtide

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.