Store at –80°C

#7445

TAOK1 Kinase

☑ 5 micrograms

Cell Signaling TECHNOLOGY®

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New 03/08

This product is for *in vitro* research use only and is not intended for use in humans or animals.

Description: Purified recombinant human TAOK1 (Met1-Lys314) kinase, supplied as a GST fusion protein.

Background: TAOKs (thousand-and-one amino acids kinases) are serine/threonine kinases belonging to the Ste-ile-20 (STE20) protein kinase family. Three different TAOK isoforms have been identified to date: TAOK1, TAOK2, and TAOK3 (1-3). TAOKs play different roles upstream of mitogen-activated protein kinase (MAPK) in signaling pathways. TAOKs behave as MEK kinases (MEKKs) in activating MAP/extracellular signal-regulated protein kinase kinase (MEKs) *in vitro*, resulting in activation of the p38 stress-sensitive pathway but not the SAPK/JNK or ERK pathways (4). Recent evidence suggests that TAOKs regulate the p38-mediated responses to DNA damage and serve as intermediates in the activation of p38 by the ATM kinase (5).

Source/Purification: The GST-Kinase fusion protein was produced using a baculovirus expression system with a construct expressing human TAOK1 (Met1-Lys314) (GenBank Accession No. NM_020791) with an amino-terminal GST tag. The protein was purified by one-step affinity chromatography using glutathione-agarose.

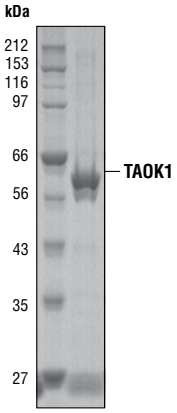

Figure 1. The purity of the TAOK1 protein was analyzed using SDS/PAGE followed by Coomassie stain.

Quality Control: The theoretical molecular weight of the GST-TAOK1 protein is 63 kDa. The purified kinase was quality controlled for purity using SDS-PAGE followed by Coomassie stain [Fig.1]. TAOK1 kinase activity was determined using a radiometric assay [Fig.2].

Background References:

(1) Hutchison, M. et al. (1998) *J Biol Chem* 273, 28625–32.

(2) Chen, Z. et al. (1999) *J Biol Chem* 274, 28803–7.

(3) Tassi, E. et al. (1999) *J Biol Chem* 274, 33287–95.

(4) Yustein, J.T. et al. (2003) *Oncogene* 22, 6129–41.

(5) Raman, M. et al. (2007) *EMBO J* 26, 2005–14.

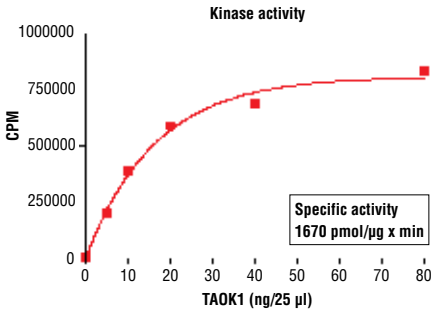

Figure 2. TAOK1 kinase activity was measured in a radiometric assay using the following reaction conditions: 5 mM MOPS, pH 7.2, 2.5 mM β-glycerophosphate, 1 mM EGTA, 0.4 mM EDTA, 5 mM $MgCl_2$, 0.05 mM DTT, 50 µM ATP, Substrate: MBP 200 ng/µl, and variable amounts of recombinant TAOK1.

Storage: Enzyme is supplied in 50 mM Tris-HCl, pH 7.5; 150 mM NaCl, 0.25 mM DTT, 0.1mM 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:

Kinase Buffer (10X) #9802

ATP (10 mM) #9804

Staurosporine #9953

Serine/Threonine Kinase Substrate Screening Kit #7400

Protocol for TAOK1 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)**
   25 mM MOPS, pH 7.2
   12.5 mM β-glycerophosphate
   5 mM EGTA
   2 mM EDTA
   25 mM $MgC1_2$
   0.25 mM DTT
2. ATP (10 mM) #9804
3. ^{32}P-γATP
4. MBP (0.5 µg/µl)

B Suggested Protocol

1. Dilute 10 mM ATP with 3X assay buffer by 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 TAOK1 protein (100 ng/µl concentration) to 10 ng/µl with 1X assay buffer followed by 2-fold serial dilutions.
5. To start the reaction combine 10 µl diluted TAOK1 kinase solution, 10 µl MBP (0.5 µg/µl), and 5 µl 0.16 µCi/µl [^{32}P] ATP solution.

Final Assay Conditions
5 mM MOPS, pH 7.2
2.5 mM β-glycerophosphate
1 mM EGTA
5 mM $MgCl_2$
0.05 mM DTT
50 µM ATP
200 ng/µL MBP

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.

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