Store at –80°C

#7376

TAOK2 Kinase

☑5 µg

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New 01/08

Description: Purified recombinant human TAOK2 (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 or STE20 protein kinase family. Three different TAOK isoforms have been identified to date: TAOK1, TAOK2, and TAOK3 (1-3). TAOK kinases 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).

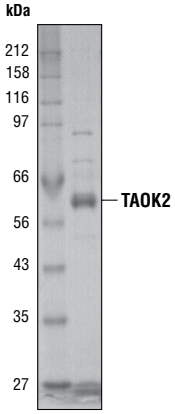

Figure 1. The purity of the TAOK2 protein was analyzed using SDS/PAGE followed by Coomassie stain.

Source/Purification: The GST-Kinase fusion protein was produced using a baculovirus expression system with a construct expressing human TAOK2 (Met1-Lys314) (GenBank Accession No. NM_004783) with an amino-terminal GST tag. The protein was purified by one-step affinity chromatography using glutathione-agarose.

Quality Control: The theoretical molecular weight of the TAOK2 protein is 63 kDa. The purified kinase was quality controlled for purity using SDS-PAGE followed by Coomassie stain [Fig.1]. TAOK2 kinase activity was determined using a radiometric assay [Fig.2].

Molecular Formula: 63000

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.

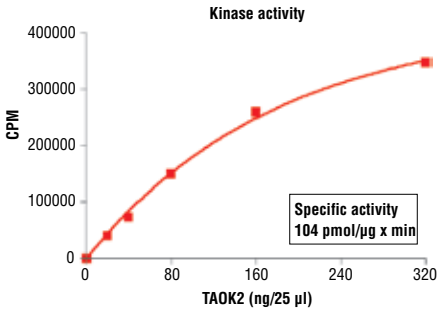

Figure 2. TAOK2 kinase activity was measured in a radiometric assay using the following reaction conditions: 5 mM MOPS, pH 7.2, 2.5 mM β-glycerophosphate, 1 mM EGTA, 0.4 mM EDTA, 5 mM $MgCl_2$, 0.05 mM DTT, 50 µM ATP, Substrate: MBP 200 ng/µl, and variable amount of recombinant TAOK2.

Storage: Enzyme is supplied in 50 mM Tris-HCl, pH7.5; 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

Serine/Threonine Kinase Substrate Screening Kit #7400

Protocol for TAOK2 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 $MgCl_2$
   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 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 TAOK2 protein (100 ng/µl concentration) to 32 ng/µl with 1X assay buffer followed by 2-fold serial dilutions.
5. To start the reaction combine 10 µl diluted TAOK2 kinase solution, 10 µl MBP (0.5 µg/µl), and 5 µl 0.16 µCi/µl [32] 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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