Store at -80°C

#7436

GSK-3β Kinase

☑5 µg

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new 07/06

Description: Purified recombinant full length human GSK-3β (Met1-Thr433) kinase, supplied as a GST fusion protein.

Background: Glycogen synthase kinase-3 (GSK-3) was initially identified as an enzyme that regulates glycogen synthesis in response to insulin (1). GSK-3 is a ubiquitously expressed serine/threonine protein kinase that phosphorylates and inactivates glycogen synthase. GSK-3 is a critical downstream element of the PI3 kinase/Akt cell survival pathway, and its activity can be inhibited by Akt-mediated phosphorylation at Ser21 of GSK-3α and Ser9 of GSK-3β (2,3). GSK-3 has been implicated in the regulation of cell fate in Dictyostelium, and is a component of the Wnt signaling pathway required for *Drosophila, Xenopus* and mammalian development (4). GSK-3 has been shown to regulate cyclin D1 proteolysis and subcellular localization (5).

Source/Purification: The GST-Kinase fusion protein was produced using a baculovirus expression system with a construct expressing human GSK-3β (Met1-Thr433) (GenBank Accession No. NM_002093) 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 GST-GSK-3β fusion protein is 73 kDa. The purified kinase was quality controlled for purity using SDS-PAGE followed by Coomassie stain [Fig.1]. GSK-3β kinase activity was determined using a radiometric assay [Fig.2].

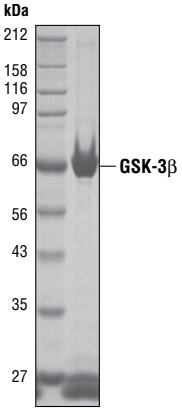

Figure 1. The purity of the GST-GSK-3β fusion protein was analyzed using SDS/PAGE followed by Coomassie stain.

Background References:

(1) Welsh, G.I. et al. (1996) *Trends Cell Biol.* 6, 274–279.

(2) Srivastava, A.K. and Pandey, S.K. (1998) *Mol. Cell. Biochem.* 182, 135–141.

(3) Cross, D.A. et al. (1995) *Nature* 378, 785–789.

(4) Nusse, R. (1997) *Cell* 89, 321–323.

(5) Diehl, J.A. et al. (1998) *Genes Dev.* 12, 3499–3511.

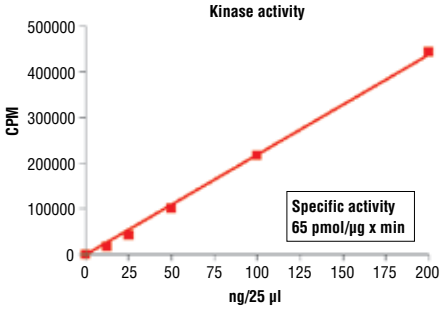

Figure 2. GSK-3β kinase activity was measured in a radiometric assay using the following reaction conditions: 4 mM MOPS, pH 7.2, 2.5 mM β-glycerophosphate, 1 mM EGTA, 0.4 mM EDTA, 4 mM $MgCl_2$, 0.05 mM DTT, 40 ng/µl BSA, 50 µM ATP, Substrate: GSK3 substrate peptide 400 ng/µL and recombinant GSK-3β: variable.

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.

Protocol for GSK-3β 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 (10X)**
   50 mM MOPS, pH 7.2
   25 mM β-glycerophosphate
   10 mM EGTA
   4 mM EDTA
   40 mM $MgC1_2$
   0.5 mM DTT
   400 ng/µl BSA
2. ATP (10 mM) #9804
3. ^{32}P-γATP
4. GSK substrate (YRRAAVPPSPSLSRHSSPHQS*EDEEE) (1 mg/ml)

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 GSK-3β protein (100 ng/µl concentration) to 20 ng/µl with 1X assay buffer followed by 2-fold serial dilutions.
5. To start the reaction combine 10 µl diluted GSK-3β kinase solution, 10 µl GSK3 substrate peptide (1 µg/µl) and 5 µl 0.16 µCi/µl [^{32}p] ATP solution.

Final Assay Conditions
4 mM MOPS, pH 7.2
2.5 mM β-glycerophosphate
1 mM EGTA
0.4 mM EDTA
4 mM $MgCl_2$
0.05 mM DTT
40 µM BSA
400 ng/µl GSK substrate

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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