Recombinant Human Active GSK3β

Catalog Number: 2506-KS-010

Lot Number: 1789156

SPECIFICATIONS AND USE

Source	Recombinant full-length human GSK3β was expressed by baculovirus in *Sf* 9 insect cells using an N terminal GST tag. The gene accession number is NM_002093.
Molecular Mass	The approximate molecular weight is 73 kDa.
Purity	The purity was determined to be > 70% by densitometry.
Formulation	Supplied in 50 mM Tris-HCl, pH 7.5, 150 mM NaCl, 0.25 mM DTT, 10 mM Glutathione, 0.1 mM EDTA, 0.1 mM PMSF, 25% Glycerol.
Size	10 µg
Concentration	0.1 µg/µL
Activity	The specific activity of GSK3β was determined to be 224 nmol/min/mg using a synthetic peptide substrate (YRRAAVPPSPSLSRHSSPHQ(pS)EDEEE).
Storage	This product is stable at ≤ -70 °C for up to one year from the date of receipt. For optimal storage, aliquot into smaller quantities after centrifugation and store at recommended temperature. **Avoid repeated freeze-thaw cycles.**

BACKGROUND

GSK3β is a Serine/Threonine protein kinase that was originally identified as the kinase that phosphorylates and inhibits glycogen synthase (1). GSK3β is ubiquitously present in human tissues and implicated in the regulation of several physiological processes, including the control of glycogen and protein synthesis by insulin and modulation of the transcription factors AP-1 and CREB. Transient transfection of human GSK3β into Chinese hamster ovary cells stably transfected with individual human tau isoforms leads to hyperphosphorylation of tau at all the sites investigated with phosphorylation-dependent anti-tau antibodies (2).

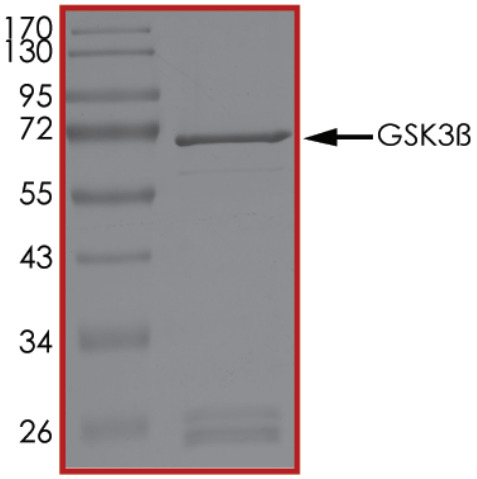

Figure 1: The purity of GSK3β was determined to be >70% by densitometry. Approximately MW 73kDa.

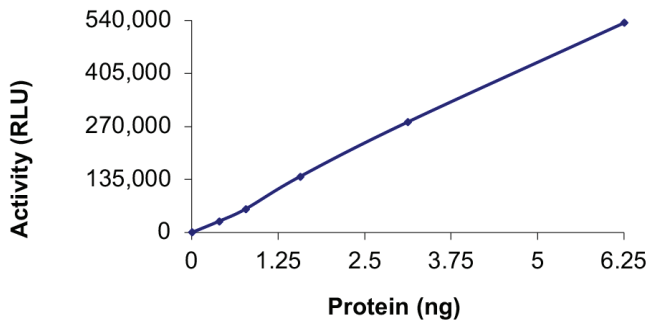

Figure 2: The specific activity of GSK3β was determined to be 224 nmol/min/mg as per Activity Assay Protocol, and was equivalent to 201 nmol/min/mg as per radiometric assay.

REFERENCES

1. Sutherland, C. *et al.* (1993) Biochem. J. **296**:15.
2. Sperber, B.R. *et al.* (1995) Neurosci. Lett. **197**:149

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ACTIVITY ASSAY PROTOCOL

Solutions Required

Active Kinase - Active GSK3β (0.1 µg/µL) diluted with Kinase Dilution Buffer 1X (1X) and assayed as outlined in Figure 2.
Note: *These are suggested working dilutions. Optimal dilutions should be determined by each laboratory for each application.*

Kinase Assay Buffer III (5X) - 200 mM Tris-HCl, pH 7.4, 100 mM $MgCl_2$, and 0.5 mg/mL BSA. Add fresh DTT prior to use to a final concentration of 250 µM.

Kinase Dilution Buffer IX (1X) - Kinase Assay Buffer III diluted at a 1:4 ratio (5X dilution) with cold distilled water. Add fresh DTT prior to use to a final concentration of 50 µM.

ADP-Glo™ Kinase Assay Kit - 10 mM ATP Solution, 10 mM ADP Solution, ADP-Glo™ Reagent, Kinase Detection Reagent.

Substrate - GSK3 synthetic peptide substrate (YRRAAVPPSPSLSRHSSPHQ(pS)EDEEE) diluted in distilled or deionized water to a final concentration of 1 mg/mL.

ASSAY PROCEDURE

The GSK3β, assay is preformed using the ADP-Glo Kinase Assay Kit, which quantifies the amount of ADP produced by the NIK reaction. The ADP-Glo Reagent is added to terminate the kinase reaction and to deplete the remaining ATP, and then the Kinase Detection Reagent is added to convert ADP to ATP and to measure the newly synthesized ATP using luciferase/luciferin reaction.

1. Thaw the Active GSK3β, Kinase Assay Buffer lll (5x), and Substrate on ice. Prepare a 15 µL enzyme dilution at the desired concentration, with Kinase Dilution Buffer IX (1X), in a pre-chilled 96-well plate.
2. Prepare a substrate/ATP mixture as follows (25 µM example):

Reaction Component	Amount
10 mM ATP Solution	1 µL
Kinase Assay Buffer III (5x)	79 µL
Substrate at 1 mg/mL	80 µL

3. Transfer the following reaction components prepared in step 2 to a 384-well opaque plate bringing the reaction volume up to 5 µL:

Component 1	3 µL of diluted Active GSK3β.
Component 2	2 µL of Substrate/ATP mix as prepared in the table above. This initiates the reaction.

4. Set up the blank control as outlined in step 2, excluding the addition of the kinase. Replace the kinase with an equal volume of Kinase Dilution Buffer IX (1x).
5. Incubate at ambient temperature for 40 minutes.
6. After the 40-minute incubation period, terminate the reaction and deplete the remaining ATP by adding 5 µL of ADP-Glo Reagent. Spin down and shake the 384-well plate. Then incubate the reaction mixture for another 40 minutes at ambient temperature.
7. Add 10 µL of the Kinase Detection Reagent to the 384-well plate and incubate the reaction mixture for another 30 minutes at ambient temperature.
8. Read the 384-well reaction plate using the Luminescence Module Protocol on a GloMax®-Multi Microplate Multimode Reader.
9. Determine the corrected activity (RLU) by removing the blank control value (see step 4) for each sample and calculate the kinase specific activity as outlined below.

Calculation of Specific Activity of ADP (RLU/pmol)

From ATP-ADP conversion curve, determine RLU/pmol of ADP

Kinase Specific Activity (SA) (pmol/min/µg or nmol/min/mg)

Corrected RLU from reaction / [(SA of ADP in RLU/pmol) * (Reaction time in min) * (Enzyme amount in µg or mg)]