

Product: GSK3b, Active

Catalog #: 02-2012

Amount: 5 mg

Product Description

Recombinant full-length human GSK3b was expressed by baculovirus in Sf9 insect cells using a N-terminal GST tag. The gene accession number is [NM_002093](#).

Gene Aliases

GPRK5

Formulation

Recombinant protein stored in 50mM Tris-HCl, pH 7.5, 150mM NaCl, 0.25mM DTT, 0.1mM EGTA, 0.1mM EDTA, 0.1mM PMSF, 25% glycerol.

Storage and Stability

Store product at -70°C. For optimal storage, aliquot target into smaller quantities after centrifugation and store at recommended temperature. For most favorable performance, avoid repeated handling and multiple freeze/thaw cycles.

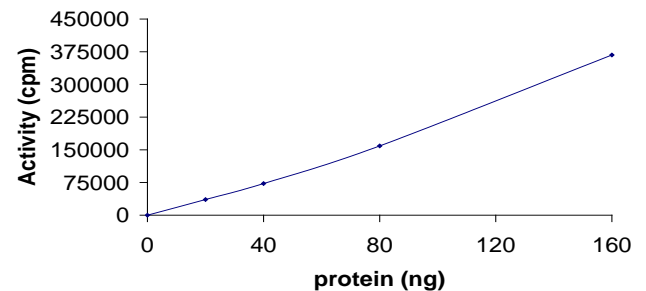
Scientific Background

GSK3b is a serine threonine protein kinase that was originally identified as the kinase that phosphorylate and inhibits glycogen synthase (1). GSK3b 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, modulation of the transcription factors AP-1 and CREB. Transient transfection of human GSK3 beta 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).

References

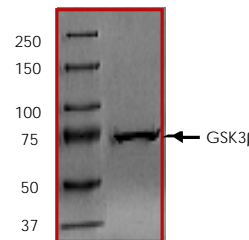
1. Sutherland C. et al: Inactivation of glycogen synthase kinase-3 beta by phosphorylation: new kinase connections in insulin and growth-factor signalling. *Biochem J.* 1993 Nov 15;296 (Pt 1):15-9.
2. Sperber BR, Leight S, Goedert M, Lee VM. Glycogen synthase kinase-3 beta phosphorylates tau protein at multiple sites in intact cells. *Neurosci Lett.* 1995 Sep 8;197(2):149-53.

Specific Activity



The specific activity of GSK3b was determined to be **101 nmol /min/mg** as per activity assay protocol.

Purity



The purity was determined to be **>90%** by densitometry. Approx. MW **73kDa**.

GSK3b, Active

Full-length recombinant protein expressed in Sf9 cells

Catalog Number **02-2012**
 Quantity **5µg**
 Specific Activity **101 nmol/min/mg**
 Specific Lot Number **B224-1**

Purity **>90%**
 Format **5µg in 50µl**
 Concentration **0.1µg/µl**
 Stability **1yr At -70°C from date of shipment**
 Storage & Shipping **Store product at -70°C. For optimal storage, aliquot target into smaller quantities after centrifugation and store at recommended temperature. For most favorable performance, avoid repeated handling and multiple freeze/thaw cycles. Product shipped on dry ice.**

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Activity Assay Protocol

Reaction Components

Active Kinase (Catalog #: 02-2012)

Active GSK3b (0.1µg/µl) diluted with Kinase Dilution Buffer (Catalog #: K23-09) and assayed as outlined in sample activity plot. (Note: these are suggested working dilutions and it is recommended that the researcher perform a serial dilution of Active GSK3b for optimal results).

Kinase Dilution Buffer, pH 7.2 (Catalog #: K23-09)

Kinase Assay Buffer I (Catalog #: K01-09) diluted at a 1:4 ratio (5X dilution) with 50 ng/µl BSA solution.

Kinase Assay Buffer I, pH 7.2 (Catalog #: K01-09)

Buffer components: 25mM MOPS, 12.5mM β-glycerol-phosphate, 25mM , 5mM EGTA, 2mM EDTA. Add 0.25mM DTT to Kinase Assay Buffer prior to use.

[γ]-ATP Assay Cocktail

Prepare 250µM [γ]-ATP Assay Cocktail in a designated radioactive working area by adding the following components: 150µl of 10mM ATP Stock Solution (Catalog #: A50-09), 100µl [³²P]-ATP (1mCi/100µl), 5.75ml of Kinase Assay Buffer (Catalog #: K01-09). Store 1ml aliquots at -20°C.

10mM ATP Stock Solution (Catalog #: A50-09)

Prepare ATP stock solution by dissolving 55mg of ATP in 10ml of Kinase Assay Buffer (Catalog #: K01-09). Store 200µl aliquots at -.

Substrate

GSK synthetic peptide substrate (YRRAAVPPSPSLSRHSSPHQ(pS)EDEEE) diluted in distilled to a final concentration of 1mg/ml.

Assay Protocol

- Step 1. Thaw [³²P]-ATP Assay Cocktail in shielded container in a designated radioactive working area.
- Step 2. Thaw the Active GSK3b, Kinase Assay Buffer, Substrate and Enzyme Dilution Buffer on ice.
- Step 3. In a pre-cooled microfuge tube, add the following reaction components bringing the initial reaction volume up to 20µl:
 - Component 1. 10µl of diluted Active GSK3b (Catalog #02-2012).
 - Component 2. 10µl of 1mg/ml stock solution of substrate
- Step 4. Set up the blank control as outlined in step 3, excluding the addition of the substrate. Replace the substrate with an equal volume of distilled H₂O.
- Step 5. Initiate the reaction by the addition of 5µl [³²P]-ATP Assay Cocktail bringing the final volume up to 25µl and incubate the mixture in a water bath at 30°C for 15 minutes.
- Step 6. After the 15 minute incubation period, terminate the reaction by spotting 20µl of the reaction mixture onto individual pre-cut strips of phosphocellulose P81 paper.
- Step 7. Air dry the pre-cut P81 strip and sequentially wash in a 1% phosphoric acid solution (dilute 10ml of phosphoric acid and make a 1L solution with distilled H₂O) with constant gentle stirring. It is recommended that the strips be washed a total of 3 intervals for approximately 10 minutes each.
- Step 8. Count the radioactivity on the P81 paper in the presence of scintillation fluid in a scintillation counter.
- Step 9. Determine the corrected cpm by removing the blank control value (see Step 4) for each sample and calculate the kinase specific activity as outlined below.

Calculation of [P³²]-ATP Specific Activity (SA) (cpm/pmol)

Specific activity (SA) = cpm for 5 µl [³²P]-ATP / pmoles of ATP (in 5 µl of a 250µM ATP stock solution, i.e., 1250 pmoles)

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

Corrected cpm from reaction / [(SA of ³²P-ATP in cpm/pmol)*(Reaction time in min)*(Enzyme amount in µg or mg)]*(Reaction Volume) / (Spot Volume)]

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