3050 Spruce Street, St. Louis, MO 63103 USA
Tel: (800) 521-8956 (314) 771-5765 Fax: (800) 325-5052 (314) 771-5757
email: techservice@sial.com sigma-aldrich.com

Product Information

GSK3β, active, His-tagged, human
PRECISIO® Kinase
recombinant, expressed in *Sf*9 cells

Catalog Number **G4296**
Storage Temperature –70 °C

Product Description

GSK3β is a serine threonine protein kinase 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 sites investigated with phosphorylation-dependent anti-tau antibodies.[2]

This recombinant product was expressed by baculovirus in *Sf*9 insect cells using an N-terminal His-tag. The gene accession number is NM 002093. It is supplied in 50 mM sodium phosphate, pH 7.0, 300 mM NaCl, 150 mM imidazole, 0.1 mM PMSF, 0.2 mM DTT, and 25% glycerol.

Molecular mass: ~48 kDa

Precautions and Disclaimer

This product is for R&D use only, not for drug, household, or other uses. Please consult the Material Safety Data Sheet for information regarding hazards and safe handling practices.

Storage/Stability

The product ships on dry ice and storage at –70 °C is recommended. After opening, aliquot into smaller quantities and store at –70 °C. Avoid repeated handling and multiple freeze/thaw cycles.

Figure 1.
SDS-PAGE Gel of Typical Lot:
≥70% (SDS-PAGE, densitometry)

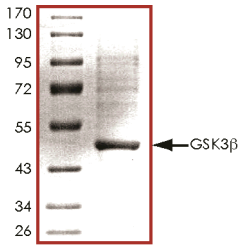
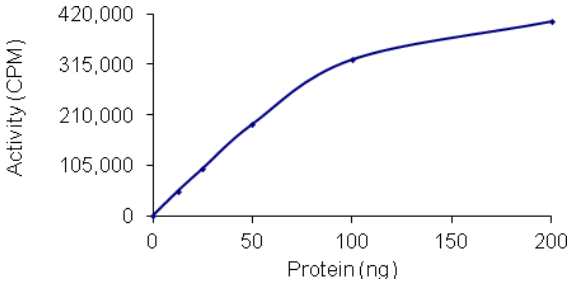


Figure 2.
Specific Activity of Typical Lot:
143–193 nmole/min/mg



Procedure

Preparation Instructions

Kinase Assay Buffer – 25 mM MOPS, pH 7.2, 12.5 mM glycerol 2-phosphate, 25 mM $MgCl_2$, 5 mM EGTA, and 2 mM EDTA. Just prior to use, add DTT to a final concentration of 0.25 mM.

Kinase Dilution Buffer – Dilute the Kinase Assay Buffer 5-fold with a 50 ng/µl BSA solution.

Kinase Solution – Dilute the active GSK3β (0.1 µg/µl) with Kinase Dilution Buffer to the desired concentration. Note: The specific activity plot may be used as a guideline (see Figure 2). It is recommended the researcher perform a serial dilution of active GSK3β kinase for optimal results.

10 mM ATP Stock Solution – Dissolve 55 mg of ATP in 10 ml of Kinase Assay Buffer. Store in 200 µl aliquots at –20 °C.

γ-^{32}P-ATP Assay Cocktail (250 µM) – Combine 5.75 ml of Kinase Assay Buffer, 150 µl of 10 mM ATP Stock Solution, 100 µl of γ-^{32}P-ATP (1 mCi/100 µl). Store in 1 ml aliquots at –20 °C.

Substrate Solution – Dissolve the synthetic peptide substrate (YRRAAVPPSPSLSRHSSPHQ(pS)EDEEE) in water at a final concentration of 1 mg/ml.

1% phosphoric acid solution – Dilute 10 ml of concentrated phosphoric acid to a final volume of 1 L with water.

Kinase Assay

This assay involves the use of the ^{32}P radioisotope. All institutional guidelines regarding the use of radioisotopes should be followed.

1. Thaw the active GSK3β, Kinase Assay Buffer, Substrate Solution, and Kinase Dilution Buffer on ice. The γ-^{32}P-ATP Assay Cocktail may be thawed at room temperature.
2. In a pre-cooled microcentrifuge tube, add the following solutions to a volume of 20 µl:
   10 µl of Kinase Solution
   10 µl of Substrate Solution
3. Set up a blank control as outlined in step 2, substituting 10 µl of cold water (4 °C) for the Substrate Solution.
4. Initiate each reaction with the addition of 5 µl of the γ-^{32}P-ATP Assay Cocktail, bringing the final reaction volume to 25 µl. Incubate the mixture in a water bath at 30 °C for 15 minutes.
5. After the 15 minute incubation, stop the reaction by spotting 20 µl of the reaction mixture onto an individually precut strip of phosphocellulose P81 paper.
6. Air dry the precut P81 strip and sequentially wash in the 1% phosphoric acid solution with constant gentle stirring. It is recommended the strips be washed a total of 3 times of ~10 minutes each.
7. Set up a radioactive control to measure the total γ-^{32}P-ATP counts introduced into the reaction. Spot 5 µl of the γ-^{32}P-ATP Assay Cocktail on a precut P81 strip. Dry the sample for 2 minutes and read the counts. Do not wash this sample.
8. Count the radioactivity on the P81 paper in the presence of scintillation fluid in a scintillation counter.
9. Determine the corrected cpm by subtracting the blank control value (see step 3) from each sample and calculate the kinase specific activity

Calculations:

1. Specific Radioactivity (SR) of ATP (cpm/nmole)

$$SR = \frac{\text{cpm of 5 µl of } \gamma\text{-}^{32}\text{P-ATP Assay Cocktail}}{\text{nmole of ATP}}$$

   cpm – value from control (step 7)
   nmole – 1.25 nmole (5 µl of 250 µM ATP Assay Cocktail)

2. Specific Kinase Activity (SA) (nmole/min/mg)

$$\text{nmole/min/mg} = \frac{\Delta\text{cpm} \times (25/20)}{SR \times E \times T}$$

SR = specific radioactivity of the ATP (cpm/nmole ATP)
Δcpm = cpm of the sample – cpm of the blank (step 3)
25 = total reaction volume
20 = spot volume
T = reaction time (minutes)
E = amount of enzyme (mg)

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., **296**, 15-9 (1993).
2. Sperber B.R. et al., Glycogen synthase kinase-3 beta phosphorylates tau protein at multiple sites in intact cells. Neurosci Lett., **197**, 149-53 (1995).

JR,JB,MAM 09/12-1