

Product Information

GSK3 α , active, GST-tagged, human PRECISIO® Kinase recombinant, expressed in Sf9 cells

Catalog Number **G8421**

Lot Number 061M0722

Storage Temperature –70 °C

Synonyms: GSK3 α

Product Description

GSK3 α is a multifunctional protein serine kinase, homologous to *Drosophila* 'shaggy' (zeste-white3) and implicated in the control of several regulatory proteins including glycogen synthase and transcription factors (e.g., JUN).¹ GSK3 α also plays a role in the WNT and PI3K signaling pathways. Alzheimer's disease is associated with increased production and aggregation of amyloid β -40 and 42 peptides into plaques. GSK3 α is required for maximal production of the amyloid β -40 and 42 peptides generated from the amyloid precursor protein by presenilin-dependent γ -secretase cleavage. *In vitro*, lithium, a GSK3 α inhibitor, blocks the production of the amyloid β peptides by interfering with the γ -secretase step.²

This recombinant product was expressed by baculovirus in Sf9 insect cells using an N-terminal GST-tag. The gene accession number is NM 019884. It is supplied in 50 mM Tris-HCl, pH 7.5, with 150 mM NaCl, 0.25 mM DTT, 0.1 mM EGTA, 0.1 mM EDTA, 0.1 mM PMSF, and 25% glycerol.

Molecular mass: ~81 kDa

Purity: ≥70% (SDS-PAGE, see Figure 1)

Specific Activity: 156–212 nmole/min/mg (see Figure 2)

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 Lot Number 061M0722:
>90% (densitometry)

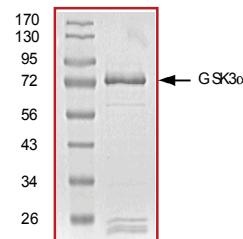
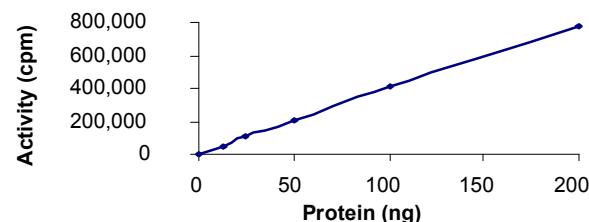


Figure 2.
Specific Activity of Lot Number 061M0722:
190 nmole/min/mg



Procedure

Preparation Instructions

Kinase Assay Buffer – 25 mM MOPS, pH 7.2, 12.5 mM glycerol 2-phosphate, 25 mM MgCl₂, 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/ μ l BSA solution.

Kinase Solution – Dilute the active GSK3 α (0.1 μ g/ μ l) with Kinase Dilution Buffer to the desired concentration. **Note:** The lot-specific specific activity plot may be used as a guideline (see Figure 2). It is recommended the researcher perform a serial dilution of active GSK3 α kinase for optimal results.

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

γ -³²P-ATP Assay Cocktail (250 μ M) – Combine 5.75 ml of Kinase Assay Buffer, 150 μ l of 10 mM ATP Stock Solution, 100 μ l of γ -³²P-ATP (1 mCi/100 μ l). 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 ³²P radioisotope. All institutional guidelines regarding the use of radioisotopes should be followed.

1. Thaw the active GSK3 α , Kinase Assay Buffer, Substrate Solution, and Kinase Dilution Buffer on ice. The γ -³²P-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 μ l:
10 μ l of Kinase Solution
5 μ l of Substrate Solution
5 μ l of cold water (4 °C)
3. Set up a blank control as outlined in step 2, substituting 5 μ l of cold water (4 °C) for the Substrate Solution.
4. Initiate each reaction with the addition of 5 μ l of the γ -³²P-ATP Assay Cocktail, bringing the final reaction volume to 25 μ l. 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 μ l of the reaction mixture onto an individually pre-cut strip of phosphocellulose P81 paper.

6. Air dry the pre-cut 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 -³²P-ATP counts introduced into the reaction. Spot 5 μ l of the γ -³²P-ATP Assay Cocktail on a pre-cut 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)

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

cpm – value from control (step 7)

nmole – 1.25 nmole (5 μ l of 250 μ M 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)

Δ cpm = 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. Ali, A. et al., Glycogen synthase kinase-3: properties, functions, and regulation. *Chem. Rev.*, **101**, 2527-2540 (2001).
2. Phiel, C.J. et al., GSK-3-alpha regulates production of Alzheimer's disease amyloid-beta peptides. *Nature*, **423**, 435-439 (2003).

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