

# Recombinant Human Active GSK-3 $\beta$

## Certificate of Analysis

Catalog Number: 2506-KS

Lot Number: 1471718

### Specifications and Use

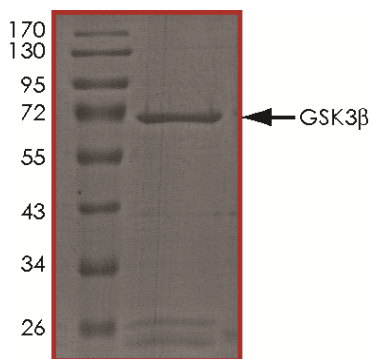
- Source** ♦ Recombinant full-length human GSK-3 $\beta$  was expressed by baculovirus in *Sf9* insect cells using an N-terminal GST tag. The gene accession number is NM\_002093.
- Molecular Mass** ♦ The approximate molecular weight is 73 kDa (see Figure 1 below).
- Purity** ♦ The purity was determined to be > 80% by densitometry (see Figure 1 below).
- 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  $\mu$ g.
- Concentration** ♦ 0.1  $\mu$ g/ $\mu$ L.
- Activity** ♦ The specific activity of GSK-3 $\beta$  was determined to be 150 nmol/min/mg using a synthetic peptide substrate (YRRAAVPPSPSLSRHSSPHQ(pS)EDEEE) (see activity assay protocol).
- Storage** ♦ This product is stable at  $\leq -70$  °C for up to 1 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.**

### GSK-3 $\beta$

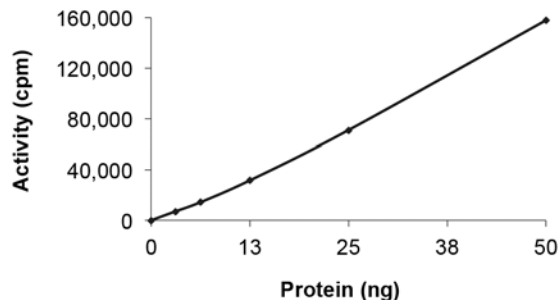
GSK-3 $\beta$  is a Serine/Threonine protein kinase that was originally identified as the kinase that phosphorylates and inhibits glycogen synthase (1). GSK-3 $\beta$  is ubiquitously present in human tissues and implicated in the regulation of several physiological processes, including the control of glycogen and protein synthesis by insulin and modulation of the transcription factors AP-1 and CREB. Transient transfection of human GSK-3 $\beta$  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.* (1993) *Biochem. J.* **296**:15.
2. Sperber, B.R. *et al.* (1995) *Neurosci. Lett.* **197**:149.



**Figure 1:** SDS-PAGE and coomassie stain. The approximate molecular weight is 73 kDa and the purity is > 80%.



**Figure 2:** Enzymatic assay results. The specific activity of GSK-3 $\beta$  was determined to be 150 nmol/min/mg as per activity assay protocol (on reverse).

FOR RESEARCH USE ONLY. NOT FOR USE IN HUMANS.

**R&D Systems, Inc.**  
**1-800-343-7475**

## Activity Assay Protocol

### Solutions Required

- **Active Kinase** - Active GSK-3 $\beta$  (0.1  $\mu\text{g}/\mu\text{L}$ ) diluted with Kinase Dilution Buffer III. **Note:** These are suggested working dilutions. Optimal dilutions should be determined by each laboratory for each application.
- **Kinase Assay Buffer I, pH 7.2** - 25 mM MOPS, 12.5 mM  $\beta$ -glycerolphosphate, 25 mM  $\text{MgCl}_2$ , 5 mM EGTA, 2 mM EDTA. Add 0.25 mM DTT to the Kinase Assay Buffer prior to use.
- **Kinase Dilution Buffer III, pH 7.2** - Kinase Assay Buffer I diluted at a 1:4 ratio (5-fold dilution) with 50  $\text{ng}/\mu\text{L}$  of BSA solution.
- **10 mM ATP Stock Solution** - Prepare the ATP Stock Solution by dissolving 55 mg of ATP in 10 mL of Kinase Assay Buffer I.
- **[ $^{33}\text{P}$ ]-ATP Assay Cocktail** - Prepare 250  $\mu\text{M}$  [ $^{33}\text{P}$ ]-ATP Assay Cocktail in a designated radioactive work area by combining 150  $\mu\text{L}$  of 10 mM ATP Stock Solution, 100  $\mu\text{L}$  of [ $^{33}\text{P}$ ]-ATP (1 mCi/100  $\mu\text{L}$ ), and 5.75 mL of Kinase Assay Buffer I.
- **Substrate** – GSK3 synthetic peptide substrate (YRRAAVPPSPSLSRHSSPHQ(pS)EDEEE) diluted in distilled or deionized water to a final concentration of 1  $\text{mg}/\text{mL}$ .

### Assay Procedure

1. Thaw the [ $^{33}\text{P}$ ]-ATP Assay Cocktail in a shielded container in a designated radioactive work area.
2. Thaw the Active GSK-3 $\beta$ , Kinase Assay Buffer I, Substrate, and Kinase Dilution Buffer III on ice.
3. In a pre-cooled microfuge tube, add the following reaction components bringing the initial reaction volume up to 20  $\mu\text{L}$ .

Reaction Component	Amount
Diluted Active GSK-3 $\beta$	10 $\mu\text{L}$
Substrate (1 $\text{mg}/\text{mL}$ Stock Solution)	5 $\mu\text{L}$
Distilled $\text{H}_2\text{O}$	5 $\mu\text{L}$

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 or deionized water.
5. Initiate the reaction by the addition of 5  $\mu\text{L}$  [ $^{33}\text{P}$ ]-ATP Assay Cocktail, bringing the final volume up to 25  $\mu\text{L}$ . Incubate the mixture in a water bath at 30  $^\circ\text{C}$  for 15 minutes.
6. After the 15-minute incubation period, terminate the reaction by spotting 20  $\mu\text{L}$  of the reaction mixture onto individual pre-cut strips of phosphocellulose P81 paper.
7. Air dry the pre-cut P81 strip and sequentially wash in a 1% phosphoric acid solution (dilute 10 mL of phosphoric acid and make a 1 liter solution with distilled or deionized water) with constant gentle stirring. It is recommended that the strips be washed a total of three times for approximately 10 minutes each.
8. Count the radioactivity on the P81 paper in the presence of scintillation fluid in a scintillation counter.
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.

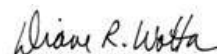
### Calculation of [ $^{33}\text{P}$ ]-ATP Specific Activity (SA) (cpm/pmol)

Specific Activity (SA) = cpm for 5  $\mu\text{L}$  [ $^{33}\text{P}$ ]-ATP/pmole of ATP (in 5  $\mu\text{L}$  of a 250  $\mu\text{M}$  ATP stock solution, *i.e.* 1250 pmoles)

### Calculation of Kinase Specific Activity (SA) (pmol/minutes/ $\mu\text{g}$ or nmol/minutes/ $\text{mg}$ )

Corrected cpm from reaction / [(SA of  $^{33}\text{P}$ -ATP in cpm/pmol) x (Reaction time in minutes) x (Enzyme amount in  $\mu\text{g}$  or  $\text{mg}$ )] x [(Reaction volume) / (Spot Volume)]

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Quality & Regulatory Affairs