

## Active Recombinant Glycogen Synthase Kinase 3 $\beta$ (GSK-3 $\beta$ )

<b>CATALOG #:</b>	7004-10	10 $\mu$ g
	7004-100	100 $\mu$ g
	7004-1000	1 mg

**LOT #:** \_\_\_\_\_

### BACKGROUND:

Glycogen synthase kinase-3 (GSK-3) is a serine/threonine kinase that was first characterized by its role in intermediary metabolism. In mammals, serine kinase is encoded by two related genes called GSK-3a (51 kDa) and GSK-3b (46 kDa). GSK-3 has been implicated in the regulation of the c-Jun/AP-1 transcription factor and other intracellular signaling pathways.

### PREPARATION:

N-terminal His-tagged human full-length recombinant GSK-3 $\beta$  was expressed in *E. coli* and purified by multiple affinity purification steps.

**FORM:** Lyophilized from 2 mg/ml in 0.1 M NH<sub>4</sub>CO<sub>3</sub>

**RECONSTITUTION:** Reconstitute to 1 mg/ml in PBS containing 15% glycerol. The solution should be stored at -70°C, stable for up to 3 months.

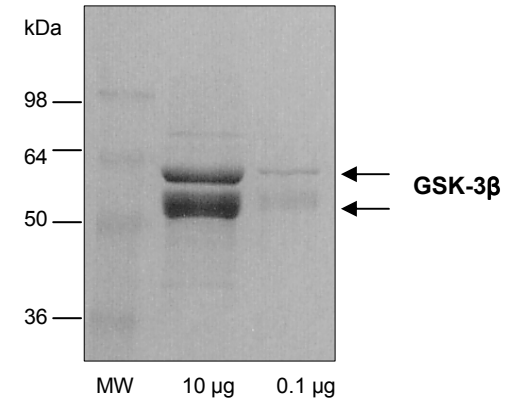
**PURITY:** >90% by SDS-PAGE

**MOL. WEIGHT:** 53-56 kDa

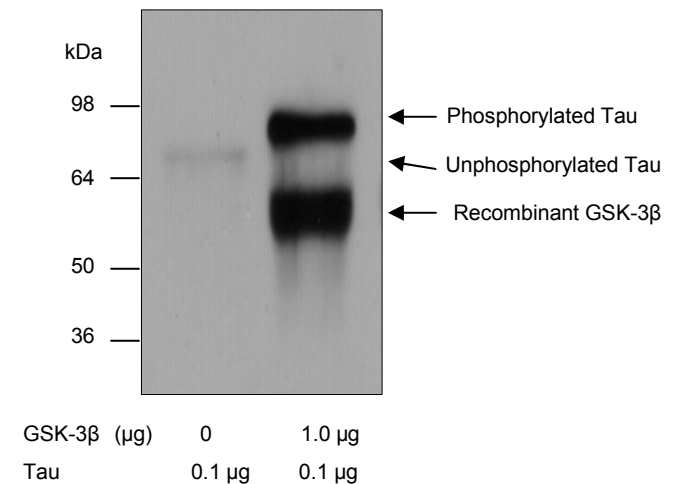
**STORAGE:** -70°C

### ACTIVITY:

Activity of GSK-3 $\beta$  was determined by an *in vitro* kinase assay using 100 ng Tau as substrate and 1  $\mu$ g of the recombinant GSK-3 $\beta$  in a 20  $\mu$ l reaction containing 25 mM Tris-HCl, pH 7.5, 5 mM  $\beta$ -glycerol phosphate, 12 mM MgCl<sub>2</sub>, 2 mM DTT, 0.1 M Na<sub>3</sub>VO<sub>4</sub>, and 200  $\mu$ M ATP. After 30 minutes incubation at 30°C, phosphorylation of Tau by the recombinant GSK-3 $\beta$  was determined by Western blot using a phospho-Tau antibody.



**Fig. 1. SDS-PAGE Stain:** Representative gel of recombinant GSK-3 $\beta$ . Arrows indicate GSK-3 $\beta$  which may be present in different phosphorylated stages.



**Fig. 2. GSK-3 $\beta$  Activity Assay Data: Analysis of Phosphorylation of Tau by recombinant GSK-3 $\beta$**  phosphorylation of Tau was determined by Western blot analysis using a phospho-Tau specific antibody. The Phospho-Tau antibody preferentially recognizes phosphorylated Tau. It also reacts with GSK-3 $\beta$  and weakly reacts with unphosphorylated Tau.

## ACTIVITY ASSAY:

Activity of GSK-3 $\beta$  can be determined by an *in vitro* kinase assay:

- 1) Add 10  $\mu$ l of Reaction Buffer (containing 25 mM Tris-HCl, pH 7.5, 5 mM  $\beta$ -glycerol phosphate, 12 mM MgCl<sub>2</sub>, 2 mM DTT, 0.1 M Na<sub>2</sub>VO<sub>4</sub>, and 200  $\mu$ M ATP) to a microcentrifuge tube.
- 2) Add 100 ng Tau (as substrate).
- 3) Add 1  $\mu$ g of the recombinant GSK-3 $\beta$
- 4) Add additional Reaction Buffer to make the final volume of 20  $\mu$ l.
- 5) Mix well and incubation for 30 minutes at 30°C.
- 6) Phosphorylation of Tau by the recombinant GSK-3 $\beta$  can be determined by standard Western blot using a phospho-Tau antibody.

**For Research Use Only! Not to be used in humans.**

## RELATED PRODUCTS:

### Apoptosis Detection Kits & Reagents

- Annexin V Kits & Bulk Reagents
- Caspase Assay Kits & Reagents
- Mitochondrial Apoptosis Kits & Reagents
- Nuclear Apoptosis Kits & Reagents
- Apoptosis Inducers and Set
- Apoptosis siRNA Vectors

### Cell Fractionation System

- Mitochondria/Cytosol Fractionation Kit
- Nuclear/Cytosol Fractionation Kit
- Membrane Protein Extraction Kit
- Cytosol/Particulate Rapid Separation Kit
- Mammalian Cell Extraction Kit
- FractionPREP Fractionation System

### Cell Proliferation & Senescence

- Quick Cell Proliferation Assay Kit
- Senescence Detection Kit
- High Throughput Apoptosis/Cell Viability Assay Kits
- LDH-Cytotoxicity Assay Kit
- Bioluminescence Cytotoxicity Assay Kit
- Live/Dead Cell Staining Kit

### Cell Damage & Repair

- HDAC Fluorometric & Colorimetric Assays & Drug Discovery Kits
- HAT Colorimetric Assay Kit & Reagents
- DNA Damage Quantification Kit
- Glutathione & Nitric Oxide Fluorometric & Colorimetric Assay Kits

### Signal Transduction

- cAMP & cGMP Assay Kits
- Akt & JNK Activity Assay Kits
- Beta-Secretase Activity Assay Kit

### Adipocyte & Lipid Transfer

- Recombinant Adiponectin, Survivin, & Leptin
- CETP Activity Assay & Drug Discovery Kits
- PLTP Activity Assay & Drug Discovery Kits
- Total Cholesterol Quantification Kit

### Molecular Biology & Reporter Assays

- siRNA Vectors
- Cloning Insert Quick Screening Kit
- Mitochondrial & Genomic DNA Isolation Kits
- 5 Minutes DNA Ligation Kit
- 20 Minutes Gel Staining/Destaining Kit
- $\beta$ -Galactosidase Staining Kit & Luciferase Reporter Assay Kit

### Growth Factors and Cytokines

### Monoclonal and Polyclonal Antibodies