

Active Recombinant Glycogen Synthase Kinase 3 β (GSK-3 β)

CATALOG #:	7004-10	10 μ g
	7004-100	100 μ 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 β was expressed in *E. coli* and purified by multiple affinity purification steps.

FORM: Lyophilized from 2 mg/ml in 0.1 M NH₄CO₃

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 β was determined by an *in vitro* kinase assay using 100 ng Tau as substrate and 1 μ g of the recombinant GSK-3 β in a 20 μ l reaction containing 25 mM Tris-HCl, pH 7.5, 5 mM β -glycerol phosphate, 12 mM MgCl₂, 2 mM DTT, 0.1 M Na₃VO₄, and 200 μ M ATP. After 30 minutes incubation at 30°C, phosphorylation of Tau by the recombinant GSK-3 β was determined by Western blot using a phospho-Tau antibody.

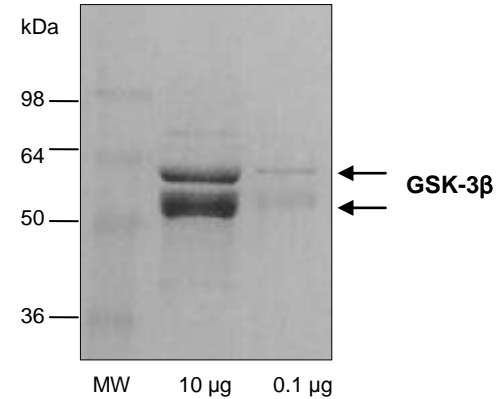


Fig. 1. SDS-PAGE Stain: Representative gel of recombinant GSK-3 β . Arrows indicate GSK-3 β which may be present in different phosphorylated stages.

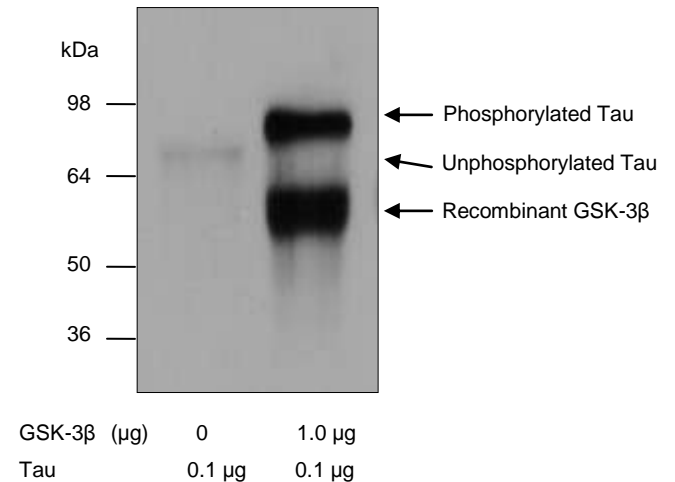


Fig. 2. GSK-3 β Activity Assay Data: Analysis of Phosphorylation of Tau by recombinant GSK-3 β phosphorylation of Tau was determined by Western blot analysis using a phospho-Tau specific antibody. The Phospho-Tau antibody preferentially recognizes phosphorylated Tau. It also reacts with GSK-3 β and weakly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

ACTIVITY ASSAY:

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

- 1) Add 10 μ l of Reaction Buffer (containing 25 mM Tris-HCl, pH 7.5, 5 mM β -glycerol phosphate, 12 mM MgCl₂, 2 mM DTT, 0.1 M Na₃VO₄, and 200 μ M ATP) to a microcentrifuge tube.
- 2) Add 100 ng Tau (as substrate).
- 3) Add 1 μ g of the recombinant GSK-3 β
- 4) Add additional Reaction Buffer to make the final volume of 20 μ l.
- 5) Mix well and incubation for 30 minutes at 30°C.
- 6) Phosphorylation of Tau by the recombinant GSK-3 β can be determined by standard Western blot using a phospho-Tau antibody.

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