

## Protein Kinase Substrate

## GSK-3 Fusion Protein

☐ Small 0.04 mg

☐ Large 0.12 mg

rev. 08/21/07

This product is for *in vitro* research use only and is not intended for use in humans or animals.
**Orders** ■ 877-616-CELL (2355)  
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**Background:** Glycogen synthase kinase-3 (GSK-3) was initially identified as an enzyme that regulated glycogen synthesis in response to insulin (1). GSK-3 is a ubiquitously expressed serine/threonine protein kinase that phosphorylates and inactivates glycogen synthase. GSK-3 is a critical downstream element of the PI3 kinase/Akt cell survival pathway, and its activity can be inhibited by Akt-mediated phosphorylation at Ser21 of GSK-3 $\alpha$  and Ser9 of GSK-3 $\beta$  (2,3). GSK-3 has been implicated in the regulation of cell fate in *Drosophila*, *Xenopus* and mammalian development (4). GSK-3 has been shown to regulate cyclin D1 proteolysis and subcellular localization (5).

**Description:** GSK-3 Fusion Protein serves as a useful substrate for assaying Akt kinase activity.

**Source/Purification:** GSK-3  $\alpha/\beta$  crosstide, corresponding to residues surrounding GSK-3  $\alpha/\beta$  (Ser21/9) (CGP-KGPGRRGRRRTSSFAEG), was fused to the N-terminus of paramyosin. The fusion protein was prepared by incubating the crosstide with bacterial-expressed paramyosin, using the IMPACT-CN System (New England Biolabs #E6900). Purified by the IMPACT-CN System (NEB #E6900).

**Quality Control:** Protein kinase substrates are examined for purity by SDS-PAGE analysis and quality control tested by Western blot using our compatible antibodies. Each substrate is also tested for its ability to be phosphorylated by its upstream kinase. This is confirmed by Western blot with the appropriate CST phospho-specific antibody.

**Directions for Use:** GSK-3 fusion protein at a concentration of 1 $\mu$ g/40 $\mu$ L reaction is phosphorylated by an upstream kinase in an *in vitro* kinase assay with 1X kinase buffer #9802 and 200 $\mu$ M ATP #9804. After a 30 minute assay at 30°C, phosphorylation can be detected using a phospho-GSK3 antibody.

**Molecular Formula:** Molecular Weight: 30 kDa

**Selected Application References:**

Cammarota, M. et al. (1999) Cyclic AMP-responsive element binding protein in brain mitochondria. *J. Neurochem.* 72, 2272–2277.

Zheng, W.H. et al. (2000) Insulin-like Growth Factor-1-induced Phosphorylation of the Forkhead Family Transcription Factor FKHL1 Is Mediated by Akt Kinase in PC12 Cells. *J. Biol. Chem.* 275, 39152–39158.

Nemoto, S. et al. (2000) Role for mitochondrial oxidants as regulators of cellular metabolism. *Mol. Cell. Biol.* 20, 7311–7318.

Ushio-Fukai, M. et al. (1999) Reactive oxygen species mediate the activation of Akt/protein kinase B by angiotensin II in vascular smooth muscle cells. *J. Biol. Chem.* 274, 22699–22704.

Peyssonnaud, C. et al. (2000) Induction of postmitotic neuroretina cell proliferation by distinct Ras downstream signaling pathways. *Mol. Cell. Biol.* 20, 7068–7079.

**Background References:**

- (1) Welsh, G.I. et al. (1996) *Trends Cell. Biol.* 6, 274–279.
- (2) Srivastava, A.K. and Pandey, S.K. (1998) *Mol. Cell. Biochem.* 182, 135–141.
- (3) Cross, D.A. et al. (1995) *Nature* 378, 785–789.
- (4) Nusse, R. (1997) *Cell* 89, 321–323.
- (5) Diehl, J.A. et al. (1998) *Genes Dev.* 12, 3499–3511.

**Storage:** Supplied in 50 mM Tris-HCl (pH 8.0 at 25°C) and 500 mM NaCl. Store at –20°C.

**Companion Products:**

Phospho-GSK-3 $\alpha/\beta$  (Ser21/9) Antibody #9331

Phospho-GSK-3 $\beta$  (Ser9) Antibody #9336

Akt Kinase Assay Kit (Nonradioactive) #9840

Akt1 Kinase #7500

