Glycogen Synthase Kinase 3 (GSK-3)

1-800-632-7799
info@neb.com
www.neb.com

P6040S 029121013101

P6040S

10,000 units 500,000 U/ml Lot: 0291210
RECOMBINANT Store at –20°C Exp: 10/13

Description: Glycogen Synthase Kinase 3 (GSK--3) is a serine/threonine protein kinase and one of several protein kinases, which phosphorylate glycogen synthase. It is also called Factor A (F_A) for its ability to activate the MgATP-dependent form of the protein phosphatase PP1 called F_C (1–4). Recent studies demonstrate that GSK-3 can autophosphorylate Ser, Thr and Tyr. Ser/Thr phosphorylation causes inactivation, and Tyr phosphorylation results in increased activity (Y216 for GSK-3β). GSK-3 expressed in *E. coli* or insect cells is extensively phosphorylated on Tyr. Molecules lacking phosphate at this position can autophosphorylate after incubation with Mg^{2+} and ATP. GSK-3 phosphorylates several exogenous substrates, but not on Tyr residues (5,6).

Recognition Determinants: The substrate specificity of GSK-3 is unique and substrate dependent. For some substrates, prior phosphorylation of the substrate to form the motif **S/T**XXXpS/pT is a strict requirement whereas in other substrates, no previous phosphorylation is needed. In either case, many of the GSK-3 sites have Pro residues close to the modified Ser or Thr (5,7).

Source: Isolated from a strain of *E. coli* that carries a clone expressing GSK-3β derived from a rabbit skeletal muscle cDNA library (kindly provided by Dr. P.J. Roach) (5).

Supplied in: 50 mM NaCl, 30 mM Tris-HCl (pH 7.5 @ 25°C), 1.0 mM EDTA, 5 mM DTT, 0.03% Brij and 50% glycerol.

Reagents Supplied with Enzyme:
10X GSK-3 Reaction Buffer

Reaction Conditions: 1X GSK-3 Reaction Buffer, supplemented with 200 µM ATP and gamma-labeled ATP to a final specific activity of 100–500 µCi/µmol. **Incubate at 30°C.**

1X GSK-3 Reaction Buffer:
20 mM Tris-HCl
10 mM $MgCl_2$
5 mM DTT
pH 7.5 @ 25°C

Note that optimal incubation times and enzyme concentrations must be determined empirically for each particular substrate.

Unit Definition: One unit is defined as the amount of GSK-3 required to catalyze the transfer of 1 pmol of phosphate to CREB Phosphopeptide, KRREIL**S**RRPpSYR (400 µM, NEB #P6041), in 1 minute at 30°C in a total reaction volume of 25 µl.

Specific Activity: ~ 5,000,000 units/mg.

Molecular Weight: 47 kDa.

Quality Assurance: GSK-3 contains no detectable protease or phosphatase activities.

Quality Control Assays

Protease Activity: After incubation of 1,000 units of Glycogen Synthase Kinase 3 (GSK-3) with 0.2 nmol of a standard mixture of proteins for 2 hours at 30°C, no proteolytic activity could be detected by SDS-PAGE analysis.

Phosphatase Activity: After incubation of 1,000 units of Glycogen Synthase Kinase 3 (GSK-3) with 50 mM *p*-nitrophenyl phosphate for 2 hours at 30°C, no phosphatase activity could be detected by spectrophotometric analysis.

(See other side)

CERTIFICATE OF ANALYSIS

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(See other side)

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References:

1. Embi, N., Rylatt, D.B. and Cohen, P. (1980) *Eur. J. Biochem*. 107, 519–527.
2. Hemmings, B.A. et al. (1982) *Eur. J. Biochem.* 119, 443–451.
3. Vandenheede, J.R. et al. (1980) *J. Biol. Chem.* 255, 11768–11774.
4. Woodgett, J.R. (1990) *EMBO J.* 9, 2431–2438.
5. Wang, Q.M. et al. (1994) *J. Biol. Chem.* 269, 14566–14574.
6. Cole, A. et al. (2004) *Biochem. J.* 377, 249–255.
7. Frame, S. and Cohen P. (2001) *Biochem. J.* 359, 1–16.

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