Glycogen Synthase Kinase 3 (GSK-3)

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 autoprophosphorylate after incubation with Mg2+ and ATP. GSK-3 phosphorylates several exogenous substrates, but not on Tyr residues (5).

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/TXXXpS/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).

Provided 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 NEBuffer for Protein Kinases

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

1X NEBuffer for Protein Kinases: 50 mM Tris-HCl
10 mM MgCl2
0.1 mM EDTA
2 mM DTT
0.01% Brij 35
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, KRRRLRPRPSYSR (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 µM p-nitrophenyl phosphate for 2 hours at 30°C, no phosphatase activity could be detected by spectrophotometric analysis.

References:

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