Molecules lacking phosphate at this position can autophosphorylate after incubation with Mg\textsuperscript{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 STXXxPS/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).

**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\textsubscript{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, KRREILRRPpSYR (400 µM, NEB #P6040), 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.
References: