### Casein Kinase I (CK1)

**Description:** Casein Kinase I (CK1) is a serine/threonine protein kinase (1). It is a truncated monomer (1–317) of the CK1b isoform, which lacks the regulatory C-terminal domain, containing 111 amino acids (2). *In vitro* studies have shown that the activity of CK1a is regulated by autophosphorylation of its C-terminal domain. Autophosphorylation of this domain on potential sites leads to inhibition of kinase activity (3). There are at least seven mammalian CK1 isoforms and their splice variants, and distinct CK1 family members have a variety of roles in eukaryotic cells (4).

**Recognition Determinants:** The most effective recognition motif for phosphorylation by CK1 is pSXXS/T where Ser in the position -3 is phosphorylated (3). Also, the clusters of 3 or 4 acidic residues ending at the position -3, preferably Asp, can specify phosphorylation by CK1. However, the substrates so formed are much poorer than those containing phosphate groups (5).

**Source:** Isolated from a strain of *E. coli* that carries a clone expressing CK1a derived from a rat testis cDNA library (kindly provided by Dr. P.J. Roach). Two codons, Ser-318 and Arg-319, have been changed to stop codons, resulting in a truncation of the C-terminal portion of the expressed protein (2).

**Reagents Supplied with Enzyme:** 10X NEBuffer for Protein Kinases

### Reaction Conditions

**Reaction Conditions:** 1X NEBuffer for Protein Kinases, supplement 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:** Note that optimal incubation times and enzyme concentrations must be determined empirically for each particular substrate.

### Quality Assurance

**Quality Assurance:** CK1 contains no detectable protease or phosphatase activities.
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