Casein Kinase I (CK1) is a serine/threonine protein kinase (1). It is a truncated monomer (1–317) of the CK16 isofrom, which lacks the regulatory C-terminal domain, containing 111 amino acids (2). In vitro studies have shown that the activity of CK16 is regulated by autophosphorylation of its C-terminal domain. The presence 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 pSXS/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 CK16 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).

**Supplied in:** 100 mM NaCl, 20 mM Tris-HCl (pH 7.0 @ 25°C), 1 mM Na2EDTA, 1 mM EGTA, 2 mM dithiothreitol, 0.1% Triton X-100 and 50% glycerol.

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

**Molecular Weight:** 36 kDa

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

**Quality Control Assays**

**Protease Activity:** After incubation of 10,000 units of Casein Kinase I (CK1) with 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 10,000 units of Casein Kinase I (CK1) with 50 mM p-nitrophenyl phosphate for 2 hours at 30°C, no phosphatase activity could be detected by spectrophotometric analysis.

**References:**

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**Recombinant Casein Kinase I (CK1) (P6030S)**

20,000 units 1,000,000 U/ml Lot: 0121504
RECOMBINANT Store at –20°C Exp: 4/16

**Description:** Casein Kinase I (CK1) is a serine/threonine protein kinase (1). It is a truncated monomer (1–317) of the CK16 isofrom, which lacks the regulatory C-terminal domain, containing 111 amino acids (2). In vitro studies have shown that the activity of CK16 is regulated by autophosphorylation of its C-terminal domain. The presence 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).

**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 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 CK1 required to catalyze the transfer of 1 pmol of phosphate to CK1 Phosphopeptide Substrate, KRRRALpSVAQPGL (70 µM, NEB #P6031), in 1 minute at 30°C in a total reaction volume of 25 µl.

**Specific Activity:** 2,000,000 units/mg

**Molecular Weight:** 36 kDa

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

**Quality Control Assays**

**Protease Activity:** After incubation of 10,000 units of Casein Kinase I (CK1) with 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 10,000 units of Casein Kinase I (CK1) with 50 mM p-nitrophenyl phosphate for 2 hours at 30°C, no phosphatase activity could be detected by spectrophotometric analysis.

**References:**