Recombinant Rat Casein Kinase I

Cat. No. CK1-245R **Lot. No.** (See product label)

SPECIFICATION

Product Overview	Recombinant Rat Casein Kinase I expressed in E. Coli.
Species	Rat
Source	E.coli
Description	Casein Kinase I (CK1) is a serine/threonine protein kinase. It is a truncated monomer (1-317) of the CK1d isoform, which lacks the regulatory C-terminal domain, containing 111 amino acids. In vitro studies have shown that the activity of CK1δ is regulated by autophosphorylation of its C-terminal domain. Autophosphorylation of this domain on potential sites leads to inhibition of kinase activity. 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.
Concentration	1000000 units/ml
MolecularWeight	36 kDa
SpecificActivity	2,000,000 units/mg
Recognition Determinants	The most effective recognition motif for phosphorylation by CK1 is pSXXS/T where Ser in the position -3 is phosphorylated. 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

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	than those containing phosphate groups.
ReactionConditions	1X CK1 Reaction Buffer. Supplemented with 200 μM ATP and 300 μCi/μmol gamma-labeled ATP. Incubate at 30°C.
1XCK1 Reaction Buffer	50 mM Tris-HCl, 10 mM MgCl2, 5 mM DTT, pH 7.5, 25°C
UnitDefinition	One unit is defined as the amount of CK1 required to catalyze the transfer of 1 pmol of phosphate to CK1 Phosphopeptide Substrate, KRRRALpSVASLPGL (70 μM), in 1 minute at 30°C in a total reaction volume of 25 μ
StorageConditions	20 mM Tris-HCl, 100 mM NaCl, 2 mM DTT, 1 mM Na2EDTA, 1 mM EGTA, 50% Glycerol, 0.1% Triton X-100, pH 7.0, 25°C
Storage	-20°C
Notes	General notes: If the source of protein to be phosphorylated is a crude extract of cells or tissue, it is very important to include the appropriate protease and protein phosphatase inhibitors in the lysis buffer and to use shorter incubation time for phosphorylation. Reaction Conditions: 1X CK1 Reaction Buffer, supplement with 200 μM ATP and gamma-labeled ATP to a final specific activity of 100-500 μCi/μmol.Usage notes: Optimal incubation times and enzyme concentrations must be determined empirically for each particular substrate. If possible, the ATP concentration should be at or near saturation (5 -10-fold over Km). Apparent Km values of ATP for most protein kinases are below 100 μM. However, if the objective is to measure enzyme activity using gamma-labeled ATP, it is best to use 100-200 μM ATP in order to have higher specific activity of gamma-labeled ATP (100-500 cpm/pmol). Also, an excess of substrate should be used, and the level of phosphorylation should not

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	exceed 10% for determination of the initial rate. To phosphorylate a protein or peptide substrate to completion, the ATP concentration should be about 5-fold over the limited substrate concentration. Higher enzyme concentration and prolonged incubation times should be employed.
QualityAssurance Statement	CK1 contains no detectable protease or phosphatase activities.
ProteaseActivity	After incubation of 10,000 units of Casein Kinase I (CK1) with a standard mixture of proteins for 2 hours at 30ºC, no proteolytic activity could be detected by SDS-PAGE.
ContaminatingPhosphatases	After incubation of 10,000 units of with 50 mM p-nitrophenyl phosphate for 2 hours at 30ºC, no phosphatase activity could be detected by spectrophotometric analysis.
Background	Introduction: The Casein kinase 1 family (EC 2.7.11.1) of protein kinases are serine/threonine-selective enzymes that function as regulators of signal transduction pathways in most eukaryotic cell types. CK1 isoforms are involved in Wnt signaling, circadian rhythms, nucleo-cytoplasmic shuttling of transcription factors, DNA repair, and DNA transcription. Keywords: casein kinase 1, alpha 1; casein kinase 1, gamma 1; casein kinase 1, gamma 2; casein kinase 1, gamma 3; casein kinase 1, delta; casein kinase 1, epsilon

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