Casein Kinase I (CK1)

1-800-632-7799
info@neb.com
www.neb.com

P6030S 012121013101

P6030S

20,000 units **1,000,000 U/ml** **Lot: 0121210**

RECOMBINANT **Store at –20°C** **Exp: 10/13**

Description: Casein Kinase I (CK1) is a serine/threonine protein kinase (1). It is a truncated monomer (1–317) of the CK1δ isoform, which lacks the regulatory C-terminal domain, containing 111 amino acids (2). *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 (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 pSXX**S/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 CK1δ 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 Na_2EDTA, 1 mM EGTA, 2 mM dithiothreitol, 0.1% Triton X-100 and 50% glycerol.

Reagents Supplied with Enzyme:
10X CK1 Reaction Buffer

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. **Incubate at 30°C.**

1X CK1 Reaction Buffer:
50 mM Tris-HCl
10 mM $MgCl_2$
5 mM dithiothreitol
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, KRRRALpSVA**S**LPGL (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:

1. Hathaway, G.M. and Traugh, J.A. (1979) *J. Biol. Chem.* 254, 762–768.
2. Graves, P.R., et al. (1993) *J. Biol. Chem.* 268, 6394–6401.
3. Graves, P.R. and Roach, P.J. (1995) *J. Biol. Chem.* 270, 21689–21694.
4. Knippschild, U. et al. (2005) *Onkologie*, 28, 508–514.
5. Flotow, H. and Poach, P.J. (1991) *J. Biol. Chem.* 266, 3724–3727.

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