Casein Kinase II (CK2)

NEW ENGLAND BioLabs Inc.
1-800-632-7799
info@neb.com
www.neb.com

P6010S 016151016101

P6010S

10,000 units 500,000 U/ml Lot: 0161510
RECOMBINANT Store at –80°C Exp: 10/16

Description: Casein Kinase II (CK2) is a constitutively active serine/threonine protein kinase composed of two 44 kDa catalytic α-subunits and two 26 kDa regulatory β-subunits in an $\alpha_2\beta_2$ configuration to form stable heterotetramers. CK2 holoenzyme undergoes autophosphorylation at two serine residues (S2/S3) of its β-subunit. Recently it has been shown that CK2 α-subunits undergo intermolecular tyrosine-autophosphorylation at Y182, which may represent a specific regulatory mechanism. Also, CK2 is able to phosphorylate, under special circumstances, tyrosyl residues in proteins. CK2 is implicated in a variety of cellular functions (1,2).

New Reaction Buffer

Recognition Determinants: The CK2 substrate specificity is invariably determined by multiple acidic residues located at positions between -2 and +5 relative to the target amino acid (mostly Ser and rarely Thr). The general recognition motif for phsophorylation by CK2 is **S**XXE/D, although **S**XE/D and **S**/D, and variations of these sequences are also phosphorylated. Polyanionic compounds, like heparin, inhibit CK2 activity with a K_i of 1.4 nm (4,5).

Source: Isolated from a strain of *E. coli* expressing both α and β CK2 subunits derived from a human glioblastoma cDNA library (kindly provided by Dr. D. Marshak) (3).

Supplied in: 350 mM NaCl, 20 mM Tris-HCl (pH 7.5 @ 25°C), 1 mM Na_2EDTA, 2 mM DTT and 0.1% Triton X-100.

Reagents Supplied with Enzyme:
10X NEBuffer for Protein Kinases

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. (CK2 will also accept GTP as a phosphoryl donor in place of ATP). **Incubate at 30°C.**

1X NEBuffer for Protein Kinases:
50 mM Tris-HCl
10 mM $MgCl_2$
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 CK2 required to catalyze the transfer of 1 pmol of phosphate to CK2 Peptide Substrate, RRRADD**S**DDDDD (100 µM, NEB #P6012), in 1 minute at 30°C in a total reaction volume of 25 µl (4,5).

Specific Activity: ~ 859,000 units/mg.

Molecular Weight: α-subunit (45 kDa), β-subunit (25 kDa). The apparent molecular weight of the α-subunit estimated by SDS-PAGE is about 42 kDa.

Quality Assurance: CK2 contains no detectable protease or phosphatase activities.

Quality Control Assays

Protease Activity: After incubation of 5,000 units of Casein Kinase II (CK2) 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 5,000 units of Casein Kinase II (CK2) with 50 mM *p*-nitrophenyl phosphate for 2 hours at 30°C, no phosphatase activity could be detected by spectrophotometric analysis.

(See other side)

CERTIFICATE OF ANALYSIS

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(See other side)

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References:

1. Donella-Deana, A. et al. (2001) *Biochem J.* 357, 563–567.
2. Marin, O. et al. (1999) *J. Biol. Chem.* 274, 29260–29265.
3. Chester, N. and Marshak, D.R. (1993) *Anal. Biochem.* 209, 284–290.
4. Marin, O. et al. (1994) *BBRC*, 198, 898–905.
5. Sarno, S. et al. (1996) *J. Biol. Chem.* 271, 10595–10601.

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