

## Casein Kinase II (CK2)



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P6010S 016140215021

# P6010S



**10,000 units 500,000 U/ml Lot: 0161402**  
**RECOMBINANT Store at -70°C Exp: 2/15**

**Description:** Casein Kinase II (CK2) is a constitutively active serine/threonine protein kinase composed of two 44 kDa catalytic  $\alpha$ -subunits and two 26 kDa regulatory  $\beta$ -subunits in an  $\alpha_2\beta_2$  configuration to form stable heterotetramers. CK2 holoenzyme undergoes autophosphorylation at two serine residues (S2/S3) of its  $\beta$ -subunit. Recently it has been shown that CK2  $\alpha$ -subunits undergo intermolecular tyrosine-autophosphorylation at Y182, which may represent a specific regulatory

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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).

**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 phosphorylation by CK2 is **SXXE/D**, although **SXE/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  $\alpha$  and  $\beta$  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<sub>2</sub>EDTA, 2 mM DTT and 0.1% Triton X-100.

**Reagents Supplied with Enzyme:**  
10X CK2 Reaction Buffer

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**Reaction Conditions:** 1X CK2 Reaction Buffer, supplement with 200  $\mu$ M ATP and gamma-labeled ATP to a final specific activity of 100–500  $\mu$ Ci/ $\mu$ mol. (CK2 will also accept GTP as a phosphoryl donor in place of ATP). **Incubate at 30°C.**

**1X CK2 Reaction Buffer:**  
20 mM Tris-HCl  
50 mM KCl  
10 mM MgCl<sub>2</sub>  
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, RRRADDSDDDDD (100  $\mu$ M, NEB #P6012), in 1 minute at 30°C in a total reaction volume of 25  $\mu$ l (4,5).

**Specific Activity:** ~ 859,000 units/mg.

**Molecular Weight:**  $\alpha$ -subunit (45 kDa),  $\beta$ -subunit (25 kDa). The apparent molecular weight of the  $\alpha$ -subunit estimated by SDS-PAGE is about 42 kDa.

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**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.

### 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.
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