

# Casein Kinase II



Technical Bulletin No. 514

INSTRUCTIONS FOR USE OF PRODUCT V5621. PLEASE DISCARD PREVIOUS VERSIONS.  
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## I. Description

Casein Kinase II (CKII) is a ubiquitous serine/threonine protein kinase found in eukaryotic cells. CKII is highly conserved among eukaryotes, including yeast, *Dictyostelium discoideum*, *C. elegans*, *Drosophila*, plants, bovines and humans. CKII is known also as phosphoinositide kinase, glycogen synthase 5 kinase, troponin T kinase and casein kinase G. The diversity in nomenclature reflects this kinase's broad range of substrates and its unique ability to utilize GTP as well as ATP as the phosphate donor. Additional names include casein kinase TS and protein kinase NII, which respectively indicate the phosphorylation targets (threonine and serine residues) and the presence of this kinase in the nucleus as well as in the cytoplasm and mitochondria. As this complicated nomenclature suggests, CKII is a multifunctional protein kinase that has been implicated in a variety of cellular processes and functions, including mitosis and cellular transformation.

Several nuclear proteins, enzymes and transcription factors serve as substrates for CKII. Phosphorylation by CKII in vitro alters the DNA binding or catalytic activity of some of these substrates, which include: c-Myc, c-Myb, serum response factor, c-Fos, c-Jun, DNA ligase, topoisomerase II, tumor suppressor proteins p53 and Rb, adenovirus E1A protein, human papilloma virus E7 protein, SV40 large T antigen, RNA polymerases I and II, nucleolar proteins B23 and C23, and several steroid hormone receptors. In addition some mitogens, including insulin, insulin-like growth factor I, epidermal growth factor, serum and phorbol esters rapidly increase the activity of CKII.

CKII isolated from most species is composed of  $\alpha$  and  $\alpha'$  subunits (37 to 44kDa) and  $\beta$  subunits (24–28kDa). The holoenzyme exists as an  $\alpha\alpha'\beta_2$  tetramer. The  $\alpha$  subunit contains the catalytic domain, whereas the  $\beta$  subunit is presumed to regulate the catalytic activity of the holoenzyme.

For additional information, please see references 1–4.



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## II. Product Components

| Product          | Size | Cat.# |
|------------------|------|-------|
| Casein Kinase II | 100u | V5621 |

Casein Kinase II is supplied frozen in a buffer containing 25mM Tris-HCl (pH 7.4), 1mM EDTA, 1mM DTT, 200mM NaCl and 50% glycerol. Please refer to the Product Information Sheet for specific information on concentration.

**Storage Conditions:** Store Casein Kinase II frozen at  $-70^{\circ}\text{C}$ , where it is stable for at least six months from the date of purchase. Avoid multiple freeze/thaw cycles. Store at  $4^{\circ}\text{C}$  if entire vial will be used within one to two weeks.

## III. Applications

- Identification of Casein Kinase II substrates and inhibitors
- Identification of putative tumor promoters and study of the enzymological effects of phosphorylation
- Determination of stoichiometry and location of phosphorylation of Casein Kinase II substrates
- Microinjection studies (5,6)

## IV. Assay Conditions

Assay CKII activity at  $37^{\circ}\text{C}$  in a 50 $\mu\text{l}$  reaction containing 25mM Tris-HCl (pH 7.4), 10mM  $\text{MgCl}_2$ , 200mM NaCl, 0.1mM ATP, 1 $\mu\text{Ci}$  [ $\gamma$ - $^{32}\text{P}$ ]ATP and 2mg/ml casein or 50-100 $\mu\text{M}$  Casein Kinase II Peptide Substrate (Cat.# V5661).

1. Start the reaction by adding the equivalent of 1 $\mu\text{l}$  of CKII. Incubate the reaction at  $37^{\circ}\text{C}$  for 10 minutes.
2. Pipet 30 $\mu\text{l}$  of the reaction onto a phosphocellulose P81 (Whatman<sup>®</sup>) filter. Immerse the filter in 200ml of 0.5%  $\text{H}_3\text{PO}_4$  solution. Shake gently for 5 minutes and pour off the wash.
3. Repeat the wash four more times.
3. Rinse the filter with a small amount of 95% ethanol.
4. Dry the filter and place in a scintillation vial. Add 3ml of scintillation fluid and count. See references 7-9 for more details.



### **CKII UTILIZES**

...Mg/ATP as well as Mg/GTP and exhibits maximal activity in the presence of 200mM NaCl (pH range: 7.0-8.5). When using the CKII Peptide Substrate (Cat.# V5661), add BSA to the reaction mixture to a final concentration of 100 $\mu\text{g/ml}$ .

## V. Miscellaneous Information

**Source:** CKII is purified from rat liver and is composed of  $\alpha$ ,  $\alpha'$  and  $\beta$  subunits in the tetrameric form  $\alpha\alpha'\beta_2$ .

**Molecular Weight:** Tetrameric CKII has a molecular weight of approximately 130-150kDa.

**Unit Definition:** One unit is defined as the amount of enzyme required to transfer 1pmol of phosphate per minute at 37°C to the partially dephosphorylated casein substrate.

**Substrates:** The enzyme phosphorylates a number of substrates, including hydrolyzed casein (Sigma Cat.# C4765), dephosphophosvitin and the Casein Kinase II Peptide Substrate Arg-Arg-Arg-Glu-Glu-Glu-Thr-Glu-Glu-Glu (Cat.# V5661).

**Activators:** CKII is activated by the naturally occurring polyamines, spermine and spermidine, and by basic polypeptides.

**Inhibitors:** Heparin is a potent CKII inhibitor: 5 $\mu$ g/ml heparin inhibits >90% of the protein kinase activity of CKII. Other CKII inhibitors include 2,3-bisphosphoglycerate, acidic polypeptides, 5,6-dibromo-1-( $\beta$ -D-ribofuranosyl)benzimidazole and 5,6-dichlorobenzimidazole.

**Purity:** CKII is >85% pure by SDS-PAGE analysis and is free of other known protein kinases.

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## VI. Related Products

| Product                            | Size | Cat.# |
|------------------------------------|------|-------|
| Casein Kinase II Peptide Substrate | 1mg  | V5661 |
| Casein Kinase I                    | 100u | V5631 |
| Casein Kinase I Peptide Substrate  | 1mg  | V7441 |

## VII. References

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