

Product Contents

Casein Kinase I:

Part No. V563A
Size 100 units

Description: Casein Kinase I (CKI) is a ubiquitous and highly conserved serine/threonine protein kinase found in eukaryotic cells. CKI is a multifunctional protein kinase that is implicated in a variety of cellular functions and processes. CKI phosphorylates several substrates in vitro such as hydrolyzed and partially dephosphorylated casein, dephosphophosvitin and peptide substrates such as Asp-Asp-Asp-Glu-Glu-Ser-Ile-Thr-Arg-Arg (Ref. 1; Casein Kinase I Peptide Substrate [Cat.# V7441]) and Asp-Asp-Asp-Val-Ala-Ser-Leu-Pro-Gly-Leu-Arg-Arg-Arg, as well as various initiation factors, amino-acyl tRNA synthetases, RNA polymerase I and II, nonhistone chromatin proteins, insulin receptor, prolactin and phosphatase inhibitor-2. CKI isolated from most species is a 35–37kDa monomer. In contrast to Casein Kinase II, CKI is not sensitive to heparin inhibition.

Enzyme Storage Buffer: 20mM Tris-HCl (pH 7.0 at 25°C), 1mM EDTA, 2mM DTT, 250mM NaCl, 1mM EGTA, 0.1% Triton® X-100 and 50% glycerol.

Unit Definition: One unit is the amount of kinase needed to transfer 1 picomole of phosphate per minute at 37°C using casein as the substrate.

Storage Conditions: Store at –20°C.

Source: *E. coli* strain expressing a recombinant CKI clone derived from a rat testis cDNA library.

Quality Control Assays

Activity: Activity is determined in a reaction mix containing 25mM Tris-HCl (pH 7.4 at 25°C), 2mg/ml casein, 10mM MgCl₂, 0.1mM ATP and 1μCi [γ -³²P]-ATP. The reaction is performed at 37°C for 5 minutes and stopped by spotting onto P-81 filters.

Concentration: See product information label for lot-specific information.

Purity: Specifications require greater than 85% purity as determined by SDS-PAGE analysis and Coomassie® stain.

References

1. Agostinis, P. *et al.* (1989) A synthetic peptide substrate specific for casein kinase I. *FEBS Lett.* **259** (1), 75–8.
2. Goueli, S.A. *et al.* (1980) A modified paper-binding procedure for the assay of nucleus-associated protein phosphokinases. *J. Pharmacol. Meth.* **3**, 235–42.
3. Casnellie, J.E. (1991) Assay of protein kinases using peptides with basic residues for phosphocellulose binding. *Meth. Enzymol.* **200**, 115–20.

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I. Assay Conditions

Assay Casein Kinase I activity at 37°C in a 50µl reaction containing 25mM Tris-HCl (pH 7.4 at 25°C), 10mM MgCl₂, 0.1mM ATP, [γ -³²P]ATP (1µCi) and 2mg/ml casein.

1. Start the reaction by adding the equivalent of 1µl of Casein Kinase I. Incubate the reaction at 37°C for 5 minutes.
2. Pipet 30µl of the reaction onto a phosphocellulose P81 (Whatman®) filter. Immerse the filter in 200ml of 0.5% H₃PO₄ 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 2 and 3 (other side) for more details.

Note: When using Casein Kinase I Peptide Substrate (Cat.# V7441), add BSA to the reaction mixture to a final concentration of 100µg/ml.

II. Related Products

Product	Size	Cat.#
Casein Kinase I Peptide Substrate	1mg	V7441
Casein Kinase II	100u	V5621
Casein Kinase II Peptide Substrate	1mg	V5661