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ProductInformation

Casein Kinase Assay Kit

Product Number **CS0400**
Storage Temperature –20 °C

TECHNICAL BULLETIN

Product Description

Casein kinases (CK) are highly conserved serine/threonine kinases, ubiquitous in eukaryotic organisms, and defined by their substrate preference for casein. They are insensitive to cyclic nucleotides, Ca^{2+}, and phospholipids. Casein kinases are grouped into two main families, type I and type II, according to their structure, specificity, and response to effectors.[1]

Casein Kinase type I (CK I) is a highly conserved multifunctional monomeric protein kinase of approximately 40 kDa that exists in multiple forms in mammalian tissues. It is present in the nucleus, cytosol, plasma membrane, and microsomes. CK I is implicated in a variety of cellular functions and processes. It appears to play a role in vesicular trafficking, DNA repair, cell cycle progression, cytokinesis, glycogen metabolism, and in viral viability. CK I phosphorylates *in vitro* several substrates other than α-casein, such as cytosolic proteins, cytoskeletal proteins, membrane associated proteins, neutral cell adhesion molecule, nuclear proteins, and proteins involved in protein synthesis.[2] *In vivo* studies showed that CK I phosphorylates proteins such as glycogen synthase, SV40 large T antigen, CREM, and p53.

Casein Kinase type II (CK II) is a tetrameric enzyme of 130-150 kDa with α2β2 structure.[3] The α subunit is catalytic and the β subunit is thought to have regulatory properties. CK II is expressed in the nucleus as well as in the cytoplasm and mitochondria. It has been implicated in a variety of cellular processes and is important for signaling pathways controlling growth division including mitosis, cellular transformation, and differentiation of cells.[3,4] Several nuclear proteins, enzymes, and transcription factors serve as substrates for CK II. It was shown that some mitogens induce the activity of CK II.[5,6]

The Casein Kinase Assay Kit provides an easy method for measuring the activity of the different casein kinases by an *in vitro* phosphorylation of α-casein. The kit includes CK I and CK II specific inhibitors[7,8] to distinguish between CK I and CK II activities in the biological sample. The kit can be used for CK activity measurement in cell lysates, tissue homogenates, column fractions, or of the purified enzyme.

Components

The kit contains reagents sufficient for 100 reactions.

Assay Buffer for Casein Kinase Activity 5x Product Code A 7853 200 mM HEPES, pH 7.5, with 650 mM KCl, 50 mM $MgCl_2$, 0.05 mM ATP, 25 mM DTT, 25 mM β-glycerophosphate, and 1 mM sodium orthovanadate	1.5 ml
ATP Solution Product Code A 7978 0.9 mM ATP	0.5 ml
Enzyme Dilution Buffer Product Code E 7405 100 mM HEPES, pH 7.5	1.7 ml
Casein Kinase Substrate Product Code C 4115 10 mg/ml α-casein in water	1 ml
Casein Kinase II Inhibitor Product Code C 4240 0.5 mg/ml heparin in water	1 ml
Casein Kinase I Inhibitor Product code C 4365 2.5 mM IC261 in DMSO	0.5 ml

Reagents and Equipment Required but Not Provided

- 6.1 N [100% (w/v)] Trichloroacetic Acid (TCA) Solution, Product Code T 0699
- Ethanol, Product Code 27,074-1
- Acetone, Product Code A 4206
- Liquid scintillation vials, general purpose, Product Code Z37,682-5
- Scintillation counter
- Whatman® chromatography paper (3 MM paper), Product Code Z27,084-9 or Z27,085-7
- γ-^{32}P-ATP, approximately 3,000 Ci/mmole, 10 mCi/ml

Precautions and Disclaimer

This product is for R&D use only, not for drug, household, or other uses. Please consult the Material Safety Data Sheet for information regarding hazards and safe handling practices.

Preparation Instructions

It is recommended to use ultrapure (17 MΩ•cm or equivalent) water when preparing the reagents.

10% TCA Solution - Take 200 ml of the 6.1 N [100% (w/v)] Trichloroacetic Acid (TCA) Solution and add it to 1.8 liters of ultrapure water and mix well.

Reaction Buffers (Assay Buffer with γ-^{32}P-ATP) – Observe all regulations regarding the handling radioactive material.

A. Reaction Buffer A used in the Standard Assay for CK Activity [Assay Buffer with γ-^{32}P-ATP (333 µM ATP)] – Prior to the experiment, thaw the Assay Buffer for Casein Kinase Activity 5x (Product Code A 7853) and the ATP Solution (Product Code A 7978). Determine the volume of Reaction Buffer A required for n+2 reactions. For each 100 µl of Assay Buffer for Casein Kinase Activity 5x, add 50 µl of the ATP solution and 1 µl of γ-^{32}P-ATP. The final ATP concentration in the assay is 100 µM.

B. Reaction Buffer B used in Inhibition Assays [Assay Buffer with γ-^{32}P-ATP (50 µM ATP)] - Prior to the experiment, thaw the Assay Buffer for Casein Kinase Activity 5x (Product Code A 7853). For each 100 µl of Assay Buffer for Casein Kinase Activity 5x, add 1 µl of γ-^{32}P-ATP. The final ATP concentration in the assay is 10 µM.

Casein Kinase II Inhibitor (heparin) - Before use dilute an aliquot of Casein Kinase II Inhibitor (Product Code C 4240) 10-fold with ultrapure water to a final concentration of 50 µg/ml. This solution can be stored at –20 °C and be used for several months.

Casein Kinase I Inhibitor (IC261) - The solution should be diluted 25-fold to a final concentration of ~100 µM. This dilution must be performed gradually in order to avoid precipitation of the IC261. Therefore, perform the following dilution steps:

a. Dilute an aliquot of the 2.5 mM solution 2-fold with ultrapure water to a concentration of 1.25 mM.
b. Dilute the 1.25 mM solution 2-fold with ultrapure water to a concentration of 0.625 mM.
c. Dilute the 0.625 mM solution 2-fold with ultrapure water to a concentration of 0.312 mM.
d. Dilute the 0.312 mM solution 3-fold with ultrapure water to a concentration of 0.104 mM.

Paper Strips for sample - Prepare paper strips for absorption of the sample. Draw a 4 x 11 cm rectangle on Whatman 3MM paper (not supplied) and cut it out. Draw a horizontal line 1 cm from the edge of the paper along the length. Mark the line at 1 cm intervals and draw vertical lines (from the marks down), thus making 1 x 3 cm tabs. Cut in between the tabs leaving 1 cm uncut, so that a 1 cm horizontal strip connects all the tabs. Number each tab for identification (1, 2, etc.).

Storage/Stability

The kit is shipped on dry ice and storage at –20 °C is recommended.

Procedure

Casein kinase activity is determined by measuring the phosphorylation of α-casein with γ-^{32}P-ATP. The phosphorylated substrate is separated from the radioactive reagent by absorption on Whatman 3MM paper. After extensive washings with the 10% TCA Solution, ethanol, and acetone, the radioactivity absorbed on the paper is counted using a scintillation counter.

$$\alpha\text{-casein} + \gamma\text{-}^{32}\text{P-ATP} \xrightarrow{\text{casein kinase}} {}^{32}\text{P-}\alpha\text{-casein} + \text{ADP}$$

A. Standard Assay for CK Activity Determination
Some chemicals/biochemicals present in crude cell extracts may interfere with CK activity.[3,4,9]
Note: The final ATP concentration in the Standard Assay is 100 mM.

1. Add the reaction components, except for Reaction Buffer A, according to the reaction scheme (Table 1). Mix well.

Table 1.
Reaction scheme for Standard Assay

Test	Dilution Buffer	Enzyme* sample	Substrate α–Casein (10 mg/ml)	Ultra-pure Water	Reaction Buffer A
Sample	------	10 µl	10 µl	15 µl	15 µl
Blank	10 µl	------	10 µl	15 µl	15 µl

***** In cases where the volume of the tested enzyme is less than 10 µl, bring the final volume to 10 µl with the Enzyme Dilution Buffer (Product Code E 7405).

2. Add Reaction Buffer A to each reaction mixture and mix.
3. Incubate the samples at 37 °C for 10-15 minutes.
4. Stop the reaction by pipetting 38 µl of the reaction mixture onto a separate tab of the Whatman 3MM paper.
5. Dry the samples spotted on the Whatman 3MM paper at room temperature for 2 minutes.
6. Wash the 3MM paper - place the 3 MM paper in an appropriate container containing the 10% TCA Solution and gently shake on a linear shaker for 5 minutes.
7. Wash the 3MM paper for 1 minute (as in step 6) with fresh 10% TCA Solution 3 more times.
8. Wash the 3 MM paper with absolute ethanol twice for 1 minute each.
9. Wash the 3 MM paper with acetone for 1 minute.
10. Dry the paper at room temperature.
11. Cut the paper tabs off and place each in a small vial, appropriate for measurement of radioactivity.
12. Count the radiation in the scintillation counter using the Cerenkov channel for 1 minute.
13. For measuring the total γ-^{32}P-ATP counts introduced into the reaction, spot 15 µl of Reaction Buffer A on Whatman 3MM paper. Dry the sample for 2 minutes and read the counts. Do not wash this sample.

B. Inhibition Assays
This procedure can be used to discriminate between CKI and CKII enzyme activities. Heparin is a specific inhibitor against CKII and the IC261 inhibitor is most potent against CK1δ, CK1ε, followed by CK1α.[8]

When determining the inhibitory potency (IC50) of a CK inhibitor, a common mechanism of action is competition with the ATP, the final ATP concentration in the Inhibition Assay reaction is 10 µM.[8] For the Standard Assay for CK Activity, the ATP final concentration is 100 µM.[8]

1. Add the reaction components, except for Reaction Buffer B, according to the reaction scheme (Table 2). Mix well.

Table 2.
Reaction scheme for Inhibition Assays

Test	Dilution Buffer	Enzyme* sample	Substrate α–Casein (10 mg/ml)	CKII Inhibitor Heparin (50 µg/ml)	CKI Inhibitor (100 µM solution)	Ultrapure Water	Reaction Buffer B
Blank	10 µl	------	10 µl	------	------	20 µl	10 µl
CK activity	------	10 µl	10 µl	------	------	20 µl	10 µl
CKII activity inhibition	------	10 µl	10 µl	10 µl	------	10 µl	10 µl
CKI activity inhibition	------	10 µl	10 µl	------	10 µl	10 µl	10 µl

* In cases where the volume of the tested enzyme is less than 10 µl, bring the final volume to 10 µl with the Enzyme Dilution Buffer (Product Code E 7405).

2. Add Reaction Buffer B to each reaction mixture and mix.
3. Continue according to steps 3-12 for the Standard Assay for CK Activity Determination (Procedure, section A).
4. For measuring the total γ-^{32}P-ATP counts introduced into the reaction, spot 10 µl of Reaction Buffer B on Whatman 3MM paper. Dry the sample for 2 minutes and read the counts. Do not wash this sample.

Calculations

A. Calculate the specific radioactivity (SR) of the ATP in cpm/nmole

- ATP concentration for Standard Assay for CK Activity Determination (section A) - 100 µM
  ATP concentration for Inhibition Assays – (section B) - 10 µM
- Reaction volume - 50 µl (0.05 ml)
- nmole of ATP per test for Standard Assay for CK Activity Determination:
  100 µM x 0.05 ml = 5 nmole
  nmole of ATP per test for Inhibition Assays:
  10 µM x 0.05 ml = 0.5 nmole

$$\text{SR (cpm/nmole)} = \frac{\text{Total cpm}}{\text{nmole ATP}}$$

2. Calculate the CK specific activity of the sample according to the formula:

$$\text{Unit (nmole/min/ml)} = \frac{\Delta\text{cpm} \times \text{dil} \times (50/38)}{\text{SR} \times \text{V} \times \text{T}}$$

SR = specific radioactivity of the ATP (cpm/nmole ATP)
Δcpm = cpm of the sample – cpm of the blank
dil = dilution factor (dilution of the original sample)
50 = total reaction volume
38 = the sample portion removed for the radioactive measurement
T = time in minutes of reaction
V = enzyme volume in ml

References

1. Tauzon, P.T., and Traugh, J.A., Casein kinase I and II - multipotential serine protein kinases: structure, function, and regulation. Adv. Second Messenger Phosphoprotein Res., **23**, 123-164 (1991).
2. Gross, S.D., and Anderson, R.A., Casein kinase I: spatial organization and positioning of a multifunctional protein kinase family. Cell Signal., **10**, 699-711 (1998).
3. Pinna, L.A., Casein kinase 2: an 'eminence grise' in cellular regulation? Biochem. Biophys. Acta, **1054**, 267-284 (1990).
4. Litchfield, D.W., and Luscher, B., Casein kinase II in signal transduction and cell cycle regulation. Mol. Cell. Biochem., **127-128**, 187-199 (1993).
5. Kuenzel, E.A., et al., Substrate specificity determinants for casein kinase II as deduced from studies with synthetic peptides. J. Biol. Chem., **262**, 9136-9140 (1987).
6. Ahmed, K., et al., Characteristics of polyamine stimulation of cyclic nucleotide-independent protein kinase reactions. Biochem. J., **232**, 767-771 (1985).
7. Hathaway, G.M., et al., Inhibition of casein kinase II by heparin. J. Biol. Chem., **255**, 8038-8041 (1980).
8. Mashhoon, N., et al., Crystal Structure of a Conformation-selective Casein Kinase-1-Inhibitor. J. Biol. Chem., **275**, 20052-20060 (2000).
9. Brockman, J.L., and Anderson, R.A., Casein kinase I is regulated by phosphatidylinositol 4,5-bisphosphate in native membranes. J. Biol. Chem., **266**, 2508-12 (1991).

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