

Product Information

Casein Kinase I Assay Kit

Product Code **CS0600**

Storage Temperature -20 °C

TECHNICAL BULLETIN

Product Description

Casein Kinase type I (CK I) is a highly conserved multifunctional monomeric protein kinase of ~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 molecules, nuclear proteins, and proteins involved in protein synthesis.^{1,2} *In vivo* studies show that CK I phosphorylates proteins such as glycogen synthase, SV40 large T antigen, CREM, and p53. The CK I family consists of multiple isoforms encoded by seven distinct genes (CK I α , β , γ 1, γ 2, γ 3, δ , and ϵ).² One of these CK I isoforms, CK I δ , has been implicated in the pathogenesis of Alzheimer's disease.^{3,4}

The Casein Kinase I Assay Kit provides an easy method for measuring the activity of CK I by an *in vitro* phosphorylation of a Casein Kinase I phosphopeptide substrate. The sequence of the peptide is based on the glycogen synthase phosphorylation region.⁵

The kit contains a CK I specific inhibitor (IC261) to ensure the specificity of the CK activity measured and a purified CK I δ enzyme to serve as a positive control. The IC261 inhibitor is most potent against isoforms CK I δ and CK-I ϵ , and less potent against CK-I α .⁶ The kit can be used for CK I activity measurement in cell lysates, tissue homogenates, column fractions, or of the purified enzyme.

Components

The kit contains reagents sufficient for 50 reactions.

- Assay Buffer for Casein Kinase Activity 5x 1.5 ml
Product Code A 7853
200 mM HEPES, pH 7.5, 650 mM KCl,
50 mM MgCl₂, 0.05 mM ATP, 25 mM DTT,
25 mM β -glycerophosphate, and
1 mM sodium orthovanadate
- ATP Solution 0.5 ml
Product Code A 7978
0.9 mM ATP
- Enzyme Dilution Buffer 1.7 ml
Product Code E 7405
100 mM HEPES, pH 7.5
- Casein Kinase I phospho substrate 0.5 mg
Product Code C 9240
(KRRRALS(p)VASLPGL)
- Casein Kinase I Inhibitor (IC261) 0.5 ml
Product Code C 4365
2.5 mM IC261 in DMSO
- Casein Kinase I δ from rat 5 μ g
Product Code C 4455
- P81 Cellulose Phosphate Squares 10 each
Product Code P 5497

Reagents and Equipment Required but Not Provided

- ~85% Phosphoric acid, Product Code 79617
- Ethanol, Product Code 27,074-1
- Acetone, Product Code 17,912-4
- Liquid scintillation vials, general purpose, Product Code Z37,682-5
- Scintillation counter
- γ -³²P-ATP, ~3,000 Ci/mmol, 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.

0.5% Phosphoric Acid Solution - Add 11.8 ml of ~85% phosphoric acid to 2 liters of ultrapure water and mix well.

Reaction Buffers (Assay Buffer + γ - 32 P-ATP) – Observe all regulations regarding handling radioactive material.

- A. Reaction Buffer A is used in the Standard Assay for CK I Activity [Assay Buffer with γ - 32 P-ATP (333 μ M 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 μ l of Assay Buffer for Casein Kinase Activity 5x, add 50 μ l of the ATP solution and 1 μ l of γ - 32 P-ATP. The final ATP concentration in the assay is 100 μ M.
- B. Reaction Buffer B is used in Inhibition Assays [Assay Buffer with γ - 32 P-ATP (50 μ M ATP)]. Prior to the experiment, thaw the Assay Buffer for Casein Kinase Activity 5x (Product Code A 7853). For each 100 μ l of Assay Buffer for Casein Kinase Activity 5X, add 1 μ l of γ - 32 P-ATP. The final ATP concentration in the assay is 10 μ M.

Casein Kinase I Inhibitor (IC261) - Before use, dilute an aliquot of Casein Kinase I Inhibitor (Product Code C 4365) 5-fold with ultrapure water to a final concentration of ~500 μ M.

Casein Kinase I Substrate – Before use, dissolve the contents of the vial in 500 μ l of ultrapure water to a final concentration of 1 mg/ml.

CK I Control – Just before the assay, dilute the Casein Kinase I δ (Product Code C 4455) 15-fold with the Enzyme Dilution Buffer (Product Code E 7405), i.e. add 5 μ l of Casein Kinase I δ to 70 μ l of the Enzyme Dilution Buffer. The CK I Control can be used to confirm the assay is performing properly.

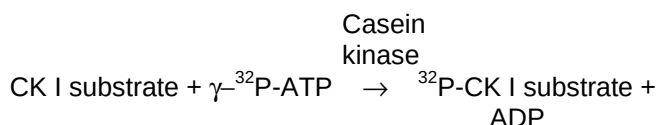
P81 Cellulose Phosphate paper is ready-to-use.

Storage/Stability

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

Procedure

Casein kinase I activity is determined by measuring the phosphorylation of the CK I peptide with γ - 32 P-ATP. The phosphorylated substrate is separated from the radioactive reagent by absorption on P81 cellulose phosphate paper squares. After extensive washings with 0.5% phosphoric acid, ethanol, and acetone, the radioactivity absorbed on the paper is counted using a scintillation counter.



A. Standard Assay for CK I Activity Determination

Some chemicals/biochemicals present in crude cell extracts may interfere with CK I activity.¹ The final ATP concentration in the Standard Assay is 100 μ M.

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	Enzyme Dilution Buffer	Enzyme* sample	CK I substrate 1 mg/ml	Ultra-pure Water	Reaction Buffer A
Sample	-----	10 μ l	10 μ l	15 μ l	15 μ l
Blank	10 μ l	-----	10 μ l	15 μ l	15 μ l

*The enzyme sample may be the CK I Control or an enzyme sample to be tested. In cases where the volume of the enzyme sample to be tested is less than 10 μ l, bring the final volume to 10 μ l 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. In parallel, number P81 cellulose phosphate paper squares according to the number of samples in the assay.
4. Stop the reaction by pipetting 38 μ l of the reaction mixture onto each P81 cellulose phosphate paper square.
5. Dry the samples spotted on the P81 cellulose phosphate paper squares at room temperature for 2 minutes.

6. Wash the P81 cellulose phosphate paper squares. Place in an appropriate container containing the 0.5% Phosphoric Acid Solution and gently shake on a linear shaker for 5 minutes.
7. Repeat step 6 three more times with fresh 0.5% Phosphoric Acid Solution.
8. Wash the P81 cellulose phosphate paper squares twice, for 1 minute each time, with absolute ethanol.
9. Wash the P81 cellulose phosphate paper squares for 1 minute with acetone.
10. Dry the paper at room temperature.
11. Cut the paper strips off and place each one in a scintillation 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 a P81 cellulose phosphate paper square. Dry the sample for 2 minutes and read the counts. Do not wash this sample.

B. Inhibition Assay

This assay can be used to verify the specificity of the CK I activity.

When determining the inhibitory potency (IC_{50}) of a CK inhibitor, where the mechanism of action is competition with the ATP, the final ATP concentration commonly used in the Inhibition Assay is 10 μM .³

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

Table 2.

Reaction Scheme for Inhibition Assay

Test	Dilution Buffer	Enzyme sample*	CK I Substrate 1 mg/ml	CK I Inhibitor IC261** (from 500 μM)	Ultrapure Water	Reaction Buffer B
Blank	10 μl	-----	10 μl	-----	20 μl	10 μl
CK I activity	-----	10 μl	10 μl	-----	20 μl	10 μl
CK-I activity inhibition	-----	10 μl	10 μl	10 μl	10 μl	10 μl

* The enzyme sample may be the CK I Control or an enzyme sample to be tested. In cases where the volume of the enzyme sample to be tested is less than 10 μl , bring the final volume to 10 μl with the Enzyme Dilution Buffer (Product Code E 7405).

** To inhibit CK I activity of a purified enzyme, the stock solution should be 5-fold less concentrated (100 μM) than that used for the inhibition of CK I activity in cell extracts (500 μM).

2. Start the reaction by the addition of the Reaction Buffer B and mix.
3. Continue according to steps 3-12 for the Standard Assay for CK I 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 a P81 cellulose phosphate paper square. Dry the sample for 2 minutes and read the counts. Do not wash this sample.

Calculations

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

- ATP concentration for Standard Assay for CK I Activity Determination (section A) - 100 μ M
- ATP concentration for Inhibition Assay (section B) - 10 μ M
- Reaction volume - 50 μ l (0.05 ml)
- nmole ATP per test for Standard Assay for CK I Activity Determination:
100 μ M x 0.05 ml = 5 nmole
- nmole ATP per test for Inhibition Assay:
10 μ M x 0.05 ml = 0.5 nmole

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

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

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

SR = specific radioactivity of the ATP (cpm/nmole ATP)

Δ cpm = 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

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3. Kuret, J., *et al.*, Casein Kinase 1 is tightly associated with paired helical filaments Isolated from Alzheimer's disease brain. *J. Neurochem.*, **69**, 2506-2515 (1997).
4. Ghoshal, N., *et al.*, A new molecular link between the Fibrillar and Granulovacuolar Lesions of Alzheimer's Disease. *Am. J. Pathol.*, **155**, 1163-1172 (1999)
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