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ProductInformation

Casein Kinase II Assay Kit

Product Code **CS 0610**
Storage Temperature –20 °C

TECHNICAL BULLETIN

Product Description

Casein Kinase type II (CK II) is a tetrameric enzyme of 130-150 kDa with $\alpha 2\beta 2$ structure.[1,2] 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.[2,3] 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.[4,5]

The Casein Kinase II Assay Kit provides an easy method for measuring enzyme activity by an *in vitro* phosphorylation of the Casein Kinase II Substrate. This substrate is a peptide that is selective for CK II with no activity with CK I. It has a very high affinity for CK II and the highest V_{max} compared to other peptide substrates.[6]

The kit also contains a CK II specific inhibitor[7] to ensure the specificity of the CK activity and in addition, it also includes purified CK II for use as a positive control. The kit can be used for CK II activity measurement in cell lysates, tissue homogenates, column fractions, or of the purified enzyme.

Components

The kit contains reagents sufficient for 70 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 II Substrate Product Code C 2460 (Arg-Arg-Arg-Ala-Asp-Asp-Ser-Asp_5)	1 mg
Casein Kinase II Inhibitor Product Code C 4240 0.5 mg/ml heparin in water	1 ml
Casein Kinase II Product Code C 3460	1 unit
P81 Cellulose Phosphate Squares Product Code P 5497	10 each

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-^{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.

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 with γ-^{32}P-ATP) – Observe all regulations regarding the handling of radioactive material.

A. Reaction Buffer A used in the Standard Assay for CK II 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 the Inhibition Assay [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 II Substrate - Before use, dissolve the contents of the vial in 700 µl of ultrapure water to a final concentration of 1 mM (1 mg in 700 µl water).

CK II Control – Just before the assay, dilute the Casein Kinase II (Product Code C 3460) 2-fold with the Enzyme Dilution Buffer (Product Code E 7405), i.e. add 5 µl of Casein Kinase II to 5 µl of the Enzyme Dilution Buffer. The CK II 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 II activity is determined by measuring the phosphorylation of the CK II substrate 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.

$$\text{CK II substrate} + \gamma\text{-}^{32}\text{P-ATP} \xrightarrow{\text{Casein Kinase II}} {}^{32}\text{P-CK II substrate} + \text{ADP}$$

A. Standard Assay for CK II Activity Determination
Some chemicals/biochemicals present in crude cell extracts may interfere with CK II activity.[2,3] The final ATP concentration in the Standard Assay is 100 µM. [8]

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 II Substrate 1 mM	Ultra-Pure Water	Reaction Buffer A
Sample	------	10 µl	10 µl	15 µl	15 µl
Blank	10 µl	------	10 µl	15 µl	15 µl

*The enzyme sample may be the CK II 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).

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 P81 cellulose phosphate paper square. For testing multiple samples, at this stage, it is not recommend to cut off the separate squares, rather handle all the samples together.
5. Dry the samples spotted on the P81 cellulose phosphate paper square at room temperature for 2 minutes.

6. Wash the P81 cellulose phosphate paper square. Place it 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 square. Place it in an appropriate container containing absolute ethanol and gently shake for 1 minute.
9. Repeat step 8 once with acetone for 1 minute.
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 II activity.

When determining the inhibitory potency (IC50) of a CK inhibitor that its mechanism of action is competition with the ATP, the final ATP concentration commonly used in the Inhibition Assay is 10 µM.

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 Assay

Test	Dilution Buffer	Enzyme* sample	CK II Substrate 1 mM	CK II Inhibitor Heparin (50 µg/ml)	Ultrapure Water	Reaction Buffer B
Blank	10 µl	------	10 µl	------	20 µl	10 µl
CK II activity	------	10 µl	10 µl	------	20 µl	10 µl
CK II activity inhibition	------	10 µl	10 µl	10 µl	10 µl	10 µl

*The enzyme sample may be the CK II 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).

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 II Activity Determination (Procedure, section A).
4. For measuring the total γ-^{32}P-ATP counts introduced into the test tube, spot 10 µl of Reaction Buffer B on a P81 cellulose phosphate paper square. Dry the sample for 2 minute 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 II Activity Determination (section A) - 100 µM
     ATP concentration for Inhibition Assay (section B) - 10 µM
   - Reaction volume - 50 µl (0.05 ml)
   - nmole ATP per test for Standard Assay for CK II Activity Determination:
     100 µM x 0.05 ml = 5 nmole
     nmole ATP per test for Inhibition Assay:
     10 µM x 0.05 ml = 0.5 nmole

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

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

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

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

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