Enzymatic Assay of CASEIN KINASE II

PRINCIPLE:

Casein + ?-^{32}P-ATP $\xrightarrow{\text{Casein Kinase II}}$ [^{32}P]-Phosphorylated Casein + ADP

CONDITIONS: T = 37°C, pH 7.5

METHOD: Radiolabelled Stop Reaction

REAGENTS:

A. 100 mM HEPES Buffer, pH 7.5 at 37°C (Enzyme Diluent)
(Prepare 25 ml in deionized water using HEPES, Free Acid, Sigma Prod. No. H-3375. Adjust to pH 7.5 at 37°C with
1 M NaOH.)

B. 200 mM HEPES Buffer with 50 mM Magnesium Chloride, 0.5 mM Adenosine 5'-Triphosphate, 650 mM Potassium Chloride, 25 mM Dithiothreitol, and 100 µCi [gamma-^{32}P] Adenosine 5-Triphosphate/ml, pH 7.5 at 37°C
(5X Reaction Buffer)
(Prepare 0.25 ml in deionized water using HEPES, Free Acid, Sigma Prod. No. H-3375, Magnesium Chloride, 4.9 M Solution, Sigma Stock No. 104-20, Dithiothreitol, Sigma Prod. No. D-9779, Potassium Chloride, Sigma Prod. No. P-4504, Adenosine 5'-Triphosphate, Disodium Salt, Sigma Prod. No. A-2383, and ?-^{32}P-Adenosine 5'-Triphosphate, 3000 Ci/mmole. Adjust to pH 7.5 at
37°C with 1 M NaOH.)

C. 1% (w/v) Casein Solution (Casein)
(Prepare 0.25 ml in deionized water using Casein, 5% Solution, Sigma Prod. No. C-4765.)

D. 50 µg/ml Heparin Solution (Heparin)
(Prepare 0.25 ml in deionized water using Heparin, Sodium, Sigma Prod. No. H-3393.)

E. 10% (w/v) Trichloroacetic Acid with 10 mM Sodium Pyrophosphate (TCA)
(Prepare 250 ml in deionized water using Trichloroacetic Acid, 6.1 N Solution, 100% (w/v), Sigma Stock No. 490-10, and Sodium Pyrophosphate, Decahydrate, Sigma Prod.

No. S-9515.)

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PROCEDURE: (continued)

F. Ethanol (EtOH)
(Use Ethyl Alcohol, Denatured, Sigma Stock No. 27,074-1)

G. Acetone
(Use Acetone, Sigma Stock No. 27,072-5.)

H. Chromatography Paper (Whatman 3mm paper)
(Use Whatman 3mm Chromatography Paper.)

I. Casein Kinase II Enzyme Solution
(Immediately before use, prepare a solution containing 0.3 unit/ml of Casein Kinase II in cold Reagent A.)

PROCEDURE:

Pipette (in milliliters) the following reagents into an Eppendorf tube.

	Test[1]	Blank
Reagent C (Casein)	0.01	0.01
Deionized Water	0.01	0.01
Reagent I (Enzyme Solution)	0.02	------
Reagent A (Enzyme Diluent)	------	0.02
Reagent B (5x Reaction Buffer)	0.01	0.01

Immediately mix by gently vortexing for a few seconds and then incubate for exactly 10 minutes at 37°C.

Pipette (in milliliter) a 0.04 ml aliquot of the Test and Blank onto a separate piece of Reagent H (Whatman 3 mm paper). Soak the pieces in Reagent E (TCA) for 2-5 minutes at room temperature.

Wash the pieces of paper 4 times with 10 ml of Reagent E (TCA). Agitate gently throughout each wash for 15 minutes. Repeat each wash 4 times with Reagent F (EtOH) and Reagent G (Acetone).

Dry the paper pieces at room temperature or under a heat lamp. Count the radioactivity incorporated into the precipitated casein using the Cerenkov method (i.e., count the ß-emission without scintillation fluid using the ^{3}H Channel).

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CALCULATIONS:

1. Count the radioactivity (R) of 0.01 ml of Reagent B (5X Reaction Buffer), in order to obtain the total radioactivity in cpm (counts per minute) per assay.

2. Divide the above value (R) by the amount of adenosine 5'-triphosphate present in the assay (5 nmole), in order to obtain the specific radioactivity (SR).

$$SR=R/5$$

3. Subtract the Blank value from the Test and multiply the result by the factor 5/4 in order to obtain the total counts per tube, C.

$$C = C_{Test} - C_{Blank} \times 5/4$$

5/4 = Correction factor since only 0.04 out of 0.05 ml reaction mix is counted.

$$\text{Units/ml enzyme} = \frac{C}{(SR)(10)(0.02)}$$

C = Radioactivity of Test in cpm
SR = Specific Radioactivity
10 = Time (in minutes) of assay
0.02 = Volume (in milliliter) of enzyme used

UNIT DEFINITION:

One unit of Casein Kinase II will transfer 1 nanomole of phosphate from ATP to casein per minute at 37°C at pH 7.5.

FINAL ASSAY CONCENTRATION:

In a 0.05 ml reaction mix, the final concentrations are 80 mM HEPES, 0.2% (w/v) casein, 10 mM magnesium chloride, 5 mM dithiothreitol, 0.1 mM adenosine 5'-triphosphate, 130 mM potassium chloride, and 0.006 unit casein kinase II.

REFERENCE:

Hathaway G.M., Lubben, T.H., and Traugh, J.A. (1980) *Journal of Biological Chemistry* **255**, 8038-8041

NOTES:

1. Run a second Test sample to be used as a control. Replace the 0.01 ml of deionized water with 0.01 ml of Reagent D (Heparin). A 10 µg/ml concentration of Heparin should inhibit the casein kinase II by more than 95%.

2. This assay is based on the cited reference.

3. Where Sigma Product or Stock numbers are specified, equivalent reagents may be substituted.