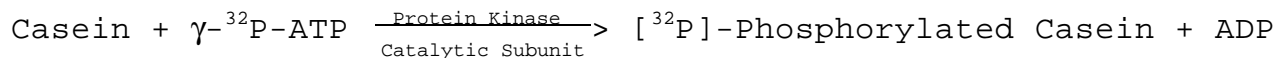


**Enzymatic Assay of PROTEIN KINASE CATALYTIC SUBUNIT
Phosphorylating Activity**

PRINCIPLE:



Abbreviations used:

$\gamma\text{}^{32}\text{P-ATP}$ = Adenosine 5'-Triphosphate $\gamma\text{}^{32}\text{P}$ label

ADP = Adenosine 5'-Diphosphate

CONDITIONS: T = 30°C, pH = 6.5

METHOD: Radioactive

REAGENTS:

- A. 1000 mM Potassium Phosphate Buffer, pH 6.5 at 30°C
(Prepare 100 ml in deionized water using Potassium Phosphate, Monobasic, Anhydrous, Sigma Prod. No. P-5379. Adjust to pH 6.5 at 30°C with 2 M KOH.)
- B. 5.0% (w/v) Casein Solution (Casein)
(Use Casein, from Bovine Milk, 5% (w/v) Solution, Sigma Prod. No. C-4765.)
- C. 500 mM Magnesium Acetate Solution ($\text{Mg}(\text{OAc})_2$)
(Prepare 10 ml in deionized water using Magnesium Acetate, Tetrahydrate, Sigma Prod. No. M-9147.)
- D. 250 mM Aminophylline Solution (AP)
(Prepare 10 ml in deionized water using Aminophylline, Hydrate, Sigma Prod. No. A-1755.)
- E. 330 mM Dithiothreitol Solution (DTT)
(Prepare 10 ml in deionized water using DL-Dithiothreitol, Sigma Prod. No. D-0632. **PREPARE FRESH.**)
- F. 10.0 mM Adenosine 5'-Triphosphate Solution (ATP)
(Prepare 1 ml in deionized water using Adenosine 5'-Triphosphate, Disodium Salt, Sigma Prod. No. A-5394. **PREPARE FRESH.**)

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REAGENTS: (continued)

- G. γ -³²P-Adenosine 5'-Triphosphate Solution (γ -³²P-ATP)
(Use product with minimum radioactive concentration of 30 curies/mole and 2 millicuries/ml.)
- H. 13.5% (w/v) Trichloroacetic Acid Solution (TCA)
(Prepare 20 ml in deionized water using Trichloroacetic Acid, 6.1 N Solution, approximately 100% (w/v), Sigma Stock No. 490-10.)
- I. Protein Kinase Catalytic Subunit Enzyme Solution
(Immediately before use, prepare a solution containing 200 - 400 units/ml of Protein Kinase Catalytic Subunit in cold Reagent J.)
- J. 39 mM DL-Dithiothreitol Solution (Enzyme Diluent)
(Prepare 10 ml in deionized water using DL-Dithiothreitol, Sigma Prod. No. D-0632. **PREPARE FRESH.**)
- K. 6.75% (w/v) Trichloroacetic Acid Solution (Wash Solution)
(Prepare 20 ml in deionized water using Trichloroacetic Acid, 6.1 N Solution, approximately 100% (w/v), Sigma Stock No. 490-10.)
- L. Methylene Cellosolve
(Prepare by adding equal volumes of Ethylene Glycol Monoethyl Ether, Sigma Prod. No. E-2632, to Ethylene Glycol Monomethyl Ether, Sigma Prod. No. E-5378.)
- M. Scintillation Cocktail
(Use Sigma-Fluor Universal LSC Cocktail for Aqueous Samples, Sigma Prod. No. S-4273.)

PROCEDURE:

Prepare a reaction cocktail by pipetting (in milliliters) the following reagents into a suitable container:

Deionized Water	2.00
Reagent A (Buffer)	0.50
Reagent C (Mg(OAc) ₂)	0.25
Reagent D (AP)	0.10
Reagent E (DTT)	0.10

Reagent F (ATP)

0.05

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PROCEDURE: (continued)

Mix by swirling. Transfer 1 ml to a suitable container and add enough Reagent G (γ - 32 P-ATP) to yield approximately 150,000-200,000 counts/minute (cpm) in 0.05 ml of the solution. Then add 0.50 ml of Reagent B (Casein). This is the Reaction Cocktail.

Pipette 0.05 ml aliquots of Reagent I (Enzyme Solution) into a multiwell disposable titerplate. Place in a 4°C ice bath.

Add 0.05 ml of the Reaction Cocktail to each well and mix by air injection. Immediately transfer the titerplate to a 30°C water bath. Incubate at 30°C for 10 minutes. Then add

0.10 ml of Reagent H (TCA) to each well.

Filter the material in the wells through 0.45 μ m Millipore HA Type filters. Wash 3 times with Reagent K (Wash Solution).

Transfer the filters to suitable 2 dram scintillation vials containing 2.00 ml of Reagent L (Methylethyl Cellosolve). To each scintillation vial, add 5 ml of Reagent M. Count the radioactivity in a suitable scintillation counter.

CALCULATIONS:

The total number of picomoles (pMoles) of ATP in the reaction mixture is calculated as follows:

$$\frac{(0.05) (0.01) (10^9)}{(3.00) (1.5)} = 1.1 \times 10^5 \text{ pmole/ml Reaction Cocktail}$$

Find **cpm/pmole** by counting 0.05 ml (5555 pmoles) of the Reaction Cocktail.

0.05 = Volume (in milliliters) of ATP used in the Reaction Cocktail

0.01 = Millimolar concentration of ATP (Reagent F)

10^9 = Conversion of millimoles to picomoles

3.00 = Intermediate volume (in milliliters) of Reaction Cocktail

1.5 = Total volume (in milliliters) of Reaction Cocktail

$$\text{Units/ml} = \frac{\text{CPM Counted}}{(\text{cpm/pmole}) (10) (0.05)}$$

CPM counted = Actual count - background on filters

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CALCULATIONS: (continued)

10 = Time of Assay (in minutes) as per the Unit Definition
0.05 = Volume (in milliliters) of Protein Kinase
Catalytic Subunit used

$$\text{Units}/\mu\text{g protein} = \frac{\text{units/ml enzyme}}{\mu\text{g protein/ml enzyme}}$$

UNIT DEFINITION:

One unit will transfer 1.0 picomole (10^{-12} mole) of phosphate from γ - ^{32}P -ATP to hydrolyzed, partially dephosphorylated casein (C-4765) per minute at pH 6.5 at 30°C.

FINAL ASSAY CONCENTRATIONS:

In a 0.10 ml solution, the final assay concentrations are 55 mM potassium phosphate, 14 mM magnesium acetate, 3 mM aminophylline, 4 mM dithiothreitol, 0.055 mM adenosine 5'-triphosphate, 0.83% (w/v) casein, and 10 - 20 units protein kinase catalytic subunit.

REFERENCES:

Riemann, E.M., Walsh, D.A., and Krebs, E.G. (1971) *Journal of Biological Chemistry* **246**, 1986-1995

Mayer, S.E., Stull, J.T., Wastila, W.B., and Thompson, B. (1974) *Methods in Enzymology*, XXXVIII, Part C, 66-73

NOTE:

1. The concentration of dithiothreitol does not include that contributed by the protein kinase catalytic subunit diluent.
2. This assay is based on the cited references.
3. Where Sigma Product or Stock numbers are specified, equivalent reagents may be substituted.

This procedure is for informational purposes. For a current copy of Sigma's quality control procedure contact our Technical Service Department.