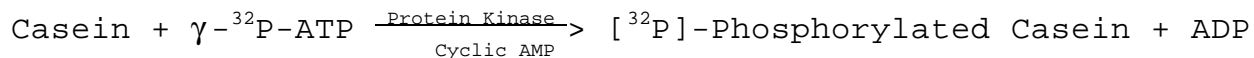


**Enzymatic Assay of PROTEIN KINASE 3':5'-CYCLIC AMP DEPENDENT
Phosphorylating Activity**

REACTION:



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 (Mg(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. 1 mM Adenosine 3':5'-Cyclic Monophosphate Solution (cAMP)
(Prepare 1 ml in deionized water using Adenosine 3':5'-Cyclic Monophosphate, Sodium Salt, Sigma Prod. No. A-6885. **PREPARE FRESH.**)

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REAGENTS: (continued)

- G. 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.**)
- H. γ -³²P-Adenosine 5'-Triphosphate Solution (γ -³²P-ATP)
(Use product with minimum radioactive concentration of 30 curies/mmol and 2 millicuries/ml.)
- I. 13.5% (v/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.)
- J. Protein Kinase 3':5'-Cyclic AMP Dependent Enzyme Solution
(Immediately before use, prepare a solution containing 200 - 400 units/ml of Protein Kinase 3':5'-Cyclic AMP Dependent in cold deionized water.)
- K. 6.75% (v/v) Trichloroacetic Acid Solution (Wash)
(Prepare 20 ml in deionized water using Trichloroacetic Acid, 6.1 N Solution, approximately 100% (w/v), Sigma Stock No. 490-10.)
- L. Methylethyl Cellosolve
(Prepare 500 ml by adding 250 ml of Ethylene Glycol Monoethyl Ether, Sigma Prod. No. E-2632, to 250 ml of 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 | 1.95 |
| Reagent A (Buffer) | 0.50 |
| Reagent C (Mg(OAc) ₂) | 0.25 |
| Reagent D (AP) | 0.10 |
| Reagent E (DTT) | 0.10 |

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PROCEDURE: (continued)

Pipette (in milliliters) the following reagents into 2.90 ml of the reaction cocktail.

| | |
|------------------|------|
| Reagent F (cAMP) | 0.05 |
| Reagent G (ATP) | 0.05 |

Mix by swirling. Transfer 1 ml to a suitable container and add enough Reagent H (γ -³²P-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 J (Enzyme Solution) into a multiwell disposable titerplate. Place in a 4°C ice bath.

Add 0.05 ml of 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 I (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).

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 (Scintillation Cocktail). 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:

$$\frac{(0.05) (0.01) (10^9)}{(3.00) (1.5)} = 1.1111 \times 10^5 \text{ pmole/ml Reaction Cocktail}$$

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 G)

10⁹ = Conversion of millimoles to picomoles

3.00 = Intermediate volume (in milliliters) of Reaction Cocktail

1.5 = Total volume (in milliliters) of Reaction Cocktail

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CALCULATION: (continued)

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

CPM counted = Actual count - background on filters
10 = Time of Assay (in minutes) as per the Unit Definition
0.05 = Volume (in milliliters) of enzyme used

UNIT DEFINITION FOR PROTEIN KINASE:

One unit will transfer 1.0 picomole (10^{-12} mole) of phosphate from γ - ^{32}P -ATP to hydrolyzed and partially dephosphorylated casein (C-4765) per minute at pH 6.5 at 30°C, in the presence of 0.006 mM cyclic-AMP (A6885).

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, 0.006 mM adenosine 3':5'-cyclic monophosphate, 0.055 mM adenosine 5'-triphosphate, 0.83% (w/v) casein, and 10 - 20 units protein kinase 3':5'-cyclic AMP dependent.

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. This assay is based on the cited references.
2. 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.