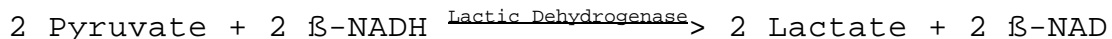
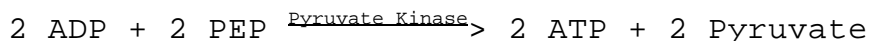
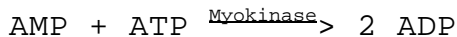
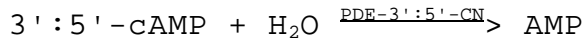


**Enzymatic Assay of PHOSPHODIESTERASE, 3':5'-CYCLIC NUCLEOTIDE
Crude Complex**

PRINCIPLE:



Abbreviations used:

3':5'-cAMP = Adenosine 3':5'-Cyclic Monophosphate

PDE-3':5'-CN = Phosphodiesterase, 3':5'-Cyclic Nucleotide

AMP = Adenosine 5'-Monophosphate

ATP = Adenosine 5'-Triphosphate

ADP = Adenosine 5'-Diphosphate

PEP = Phospho(enol)pyruvate

β -NADH = β -Nicotinamide Adenine Dinucleotide, Reduced Form

β -NAD = β -Nicotinamide Adenine Dinucleotide, Oxidized Form

CONDITIONS: T = 30°C, pH = 7.5, A_{340nm}, Light path = 1 cm

METHOD: Continuous Spectrophotometric Rate Determination

REAGENTS:

- A. 200 mM Tris HCl Buffer, pH 7.5 at 30°C
(Prepare 50 ml in deionized water using Trizma Hydrochloride, Sigma Prod. No. T-3253. Adjust to pH 7.5 at 30°C with 2 M NaOH.)
- B. 1 M Potassium Chloride Solution (KCl)
(Prepare 1 ml in deionized water using Potassium Chloride, Sigma Prod. No. P-4504.)
- C. 60 mM Magnesium Sulfate Solution (MgSO₄)
(Prepare 10 ml in deionized water using Magnesium Sulfate, Anhydrous, Sigma Prod. No. M-7506.)

**Enzymatic Assay of PHOSPHODIESTERASE, 3':5'-CYCLIC NUCLEOTIDE
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REAGENTS: (continued)

- D. 0.665 mM Phospho(enol)pyruvate Solution (PEP)
(Prepare by dissolving Phospho(enol)pyruvate,
Monopotassium Salt, Sigma Prod. No. P-7127 in 3.7 ml
of Reagent A. Then add 0.6 ml of Reagent B, 1.2 ml of
Reagent C and 4.5 ml of deionized water. **PREPARE
FRESH.**)
- E. 30 mM Adenosine 5'-Triphosphate Solution (ATP)
(Prepare 10 ml in deionized water using Adenosine
5'-Triphosphate, Disodium Salt, Sigma Prod. No. A-
5394. Adjust to pH 7.5 at 30°C with solid Sodium
Bicarbonate,
Sigma Prod. No. S-8875. **PREPARE FRESH.**)
- F. 60 mM Adenosine 3':5'-Cyclic Monophosphate Solution
(3':5'-cAMP)
(Prepare 1 ml in deionized water using
Adenosine 3':5'-Cyclic Monophosphate, Sodium Salt,
Sigma Prod. No. A-6885. **PREPARE FRESH.**)
- G. 0.70 mM Calcium Acetate Solution (Ca(OAc)₂)
(Prepare 10 ml in deionized water using Calcium
Acetate, Sigma Prod. No. C-1000.)
- H. β-Nicotinamide Adenine Dinucleotide, Reduced Form,
Prewighed vial 1 mg (β-NADH)
(Use β-Nicotinamide Adenine Dinucleotide, Reduced
Form, Disodium Salt, Sigma Stock No. 340-101. **PREPARE
FRESH.**)
- I. PK/LDH Enzymes Suspension¹
(Use PK/LDH Enzymes Suspension, Sigma Stock No. 40-7.)
- J. Myokinase Enzyme Solution (MK)
(Immediately before use, prepare a solution containing
200 units/ml using Myokinase, Sigma Prod. No. M-3003,
in cold deionized water.)
- K. Phosphodiesterase, 3':5'-Cyclic Nucleotide Crude
Complex Enzyme Solution (PDE-3':5'-CN)
(Immediately before use, prepare a solution containing
0.05 - 0.1 unit/ml of Phosphodiesterase 3':5'-Cyclic
Nucleotide, Crude Complex, in cold deionized water.)

**Enzymatic Assay of PHOSPHODIESTERASE, 3':5'-CYCLIC NUCLEOTIDE
Crude Complex**

PROCEDURE:

Prepare a reaction cocktail by pipetting (in milliliters) the following reagents into Reagent H (β -NADH):

Reagent D (PEP)	9.00
Reagent E (ATP)	0.10
Reagent F (3':5'-cAMP)	0.10
Reagent G (Ca(OAc) ₂)	0.15
Reagent I (PK/LDH)	0.05
Reagent J (MK)	0.05

Mix by inversion.

Pipette (in milliliters) the following reagents into suitable cuvettes:

	<u>Test</u>	<u>Blank</u>
Reaction Cocktail	3.00	3.00

Equilibrate to 30°C. Monitor the A_{340nm} until constant, using a suitably thermostatted spectrophotometer. Then add:

Reagent K (PDE-3':5'-CN)	0.10	-----
Deionized Water	-----	0.10

Mix by inversion and record the decrease in A_{340nm} for approximately 15 minutes. Obtain the $\Delta A_{340nm}/\text{minute}$ using the maximum linear rate for both the Test and Blank.

CALCULATIONS:

$$\text{Units/ml enzyme} = \frac{(\Delta A_{340nm}/\text{min Test} - \Delta A_{340nm}/\text{min Blank})(3.1)(df)}{(2)(6.22)(0.1)}$$

3.1 = Total volume (in milliliters) of assay

df = Dilution factor

2 = 2 μmoles of β -NAD produced per μmole of 3':5'-cAMP hydrolyzed

6.22 = Millimolar extinction coefficient of β -NADH at 340 nm

0.1 = Volume (in milliliter) of enzyme used in the assay

**Enzymatic Assay of PHOSPHODIESTERASE, 3':5'-CYCLIC NUCLEOTIDE
Crude Complex**

UNIT DEFINITION:

One unit will hydrolyze 1.0 μ mole of 3':5'-cyclic-AMP to 5'-AMP per minute at pH 7.5 at 30°C.

FINAL ASSAY CONCENTRATIONS:

In a 3.10 ml reaction mix, the final concentrations are 68 mM Tris, 55 mM potassium chloride, 7 mM magnesium sulfate, 0.61 mM phospho(enol)pyruvate, 0.31 mM adenosine 5'-triphosphate, 0.61 mM adenosine 3':5'-cyclic monophosphate, 11 units pyruvate kinase, 16 units lactic dehydrogenase, 3.1 units myokinase, 0.01 mM calcium acetate, 0.1 mM β -nicotinamide adenine dinucleotide, reduced form, and 0.005 - 0.01 unit phosphodiesterase, 3':5'-cyclic nucleotide, crude complex.

REFERENCE:

Chock, S.P. and Huang, C.Y. (1984) *Analytical Biochemistry* **138**, 34-43

NOTES:

1. Contains not less than 700 Pyruvate Kinase units and 1000 L-Lactic Dehydrogenase units per ml.
2. L-Lactic Dehydrogenase Unit Definition: One unit will reduce 1.0 μ mole of pyruvate to L-lactate per minute at pH 7.5 at 37°C.
3. Pyruvate Kinase Unit Definition: One unit will convert 1.0 μ mole of phospho(enol)pyruvate to pyruvate per minute at pH 7.6 at 37°C.
4. Myokinase Unit Definition: One unit will convert 2.0 μ moles of ADP to ATP and AMP per minute at pH 7.6 at 37°C.
5. This reaction is very sensitive to chelators, which sequester the calcium ions at low Ca^{2+} concentrations. EDTA or EGTA concentrations of 0.005 mM in the cuvette will almost completely inhibit the reaction. The concentration of EDTA or EGTA should be less than 0.001 mM in the cuvette. Cuvettes should also be completely cleaned with 3% (w/v) NaOH solution before each use, as phosphodiesterase 3':5' cyclic activator can bind to quartz or glass cuvettes.

**Enzymatic Assay of PHOSPHODIESTERASE, 3':5'-CYCLIC NUCLEOTIDE
Crude Complex**

NOTES: (continued)

6. This assay is based on the cited reference.
7. 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.