

**Enzymatic Assay of NUCLEOSIDE MONOPHOSPHATE KINASE
(EC 2.7.4.4)**

PRINCIPLE:

ATP + UMP Nucleoside Monophosphate Kinase > ADP + UDP

ADP + UDP + 2 PEP Pyruvate Kinase > 2 Pyruvate + ATP + UTP

2 Pyruvate + 2 β -NADH Lactic Dehydrogenase > 2 Lactate + 2 β -NAD

Abbreviations used:

ATP = Adenosine 5'-Triphosphate

UMP = Uridine 5'-Monophosphate

ADP = Adenosine 5'-Diphosphate

UDP = Uridine 5'-Diphosphate

PEP = Phospho(enol)pyruvate

UTP = Uridine 5'-Triphosphate

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

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

CONDITIONS: T = 25°C, pH = 7.6, A_{340nm}, Light path = 1 cm

METHOD: Continuous Spectrophotometric Rate Determination

REAGENTS:

- A. 100 mM Triethanolamine Buffer, pH 7.6 at 25°C
(Prepare 50 ml in deionized water using
Triethanolamine Hydrochloride, Sigma Prod. No. T-1502.
Adjust to pH 7.6 at 25°C with 1 N NaOH.)
- B. 27 mM Uridine 5'-Monophosphate Solution (UMP)
(Prepare 10 ml in deionized water using Uridine
5'-Monophosphate, Disodium Salt, Sigma Prod. No.
U-6375.)
- C. 18 mM Adenosine 5'-Triphosphate Solution (ATP)
(Prepare 10 ml in deionized water using Adenosine
5'-Triphosphate, Disodium Salt, Sigma Prod. No.
A-5394.)

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REAGENTS: (continued)

- D. 19 mM Phospho(enol)pyruvate Solution (PEP)
(Prepare 10 ml in deionized water using Phospho(enol)pyruvate, Mono(cyclohexylammonium) Salt, Sigma Prod. No. P-3637. **PREPARE FRESH.**)
- E. 500 mM Magnesium Sulfate with 2 M Potassium Chloride Solution (MgSO₄/KCl)
(Prepare 10 ml in deionized water using Magnesium Sulfate, Heptahydrate, Sigma Prod. No. M-1880 and Potassium Chloride, Sigma Prod. No. P-4504.)
- F. 14 mM β-Nicotinamide Adenine Dinucleotide, Reduced Form Solution (β-NADH)
(Dissolve the contents of one 10 mg vial of β-Nicotinamide Adenine Dinucleotide, Reduced Form, Disodium Salt, Sigma Stock No. 340-110, in the appropriate volume of deionized water. **PREPARE FRESH.**)
- G. PK/LDH Enzymes Suspension¹
(Use PK/LDH Enzymes Suspension, Sigma Stock No. 40-7.)
- H. Nucleoside Monophosphate Kinase Enzyme Solution.
(Immediately before use, prepare a solution containing 0.1 - 0.2 unit/ml of Nucleoside Monophosphate Kinase in cold Reagent A.)

PROCEDURE:

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

Reagent A (Buffer)	21.7
Reagent B (UMP)	1.0
Reagent C (ATP)	3.0
Reagent D (PEP)	2.0
Reagent E (MgSO ₄ /KCl)	1.0
Reagent F (β-NADH)	0.8

Mix by swirling and adjust to pH 7.6 at 25°C with 1 M HCl or 1 M NaOH, if necessary.

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PROCEDURE: (continued)

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

	Test	Blank
Reaction Cocktail	2.95	2.95
Reagent G (PK/LDH)	0.05	0.05

Mix by inversion and equilibrate to 25°C. Monitor the $A_{340\text{nm}}$ until constant, using a suitably thermostatted spectrophotometer. Then add:

Reagent A (Buffer)	-----	0.10
Reagent H (Enzyme Solution)	0.10	-----

Immediately mix by inversion and record the decrease in $A_{340\text{nm}}$ for approximately 5 minutes. Obtain the $r A_{340\text{nm}}/\text{minute}$ using the maximum linear rate for both the Test and Blank.

CALCULATIONS:

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

3.1 = Total volume (in milliliters) of assay

df = Dilution factor

2 = 2 moles of β -NAD produced per mole of ATP used

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

0.1 = Volume (in milliliters) of enzyme used

$$\text{Units/mg solid} = \frac{\text{units/ml enzyme}}{\text{mg solid/ml enzyme}}$$

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

UNIT DEFINITION:

One unit will convert 1.0 μmole of UMP and ATP to UDP and ADP per minute at pH 7.6 at 25°C, using a coupled enzyme system with PK/LDH.

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FINAL ASSAY CONCENTRATIONS:

In a 3.10 ml reaction mix, the final concentrations are 73 mM triethanolamine, 0.87 mM uridine 5'-monophosphate, 1.7 mM adenosine 5'-triphosphate, 1.2 mM phospho(enol)pyruvate, 16 mM magnesium sulfate, 65 mM potassium chloride, 0.36 mM β -nicotinamide adenine dinucleotide, reduced form, 35 units pyruvate kinase, 50 units lactic dehydrogenase and 0.01 - 0.02 unit nucleoside monophosphate kinase.

REFERENCE:

Strominger, J.L., Heppel, L.A., and Maxwell, E.S. (1959)
Biochim. Biophys. Acta **32**, 412-421.

NOTES:

1. Contains not less than 700 units/ml of Pyruvate Kinase and 1000 units/ml of Lactic Dehydrogenase.
2. 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.
3. 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.
4. This assay is based on the cited reference.
5. 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.