Enzymatic Assay of PHOSPHORIBOSYL-PYROPHOSPHATE SYNTHETASE (EC 2.7.6.1)

PRINCIPLE:

ATP + Ribose 5-Phosphate $\xrightarrow{\text{PRPP Synthetase}}$ PRPP + AMP

AMP + ATP $\xrightarrow{\text{MK}}$ 2 ADP

2 PEP + 2 ADP $\xrightarrow{\text{PK}}$ 2 Pyruvate + 2 ATP

Pyruvate + 2 ß-NADH $\xrightarrow{\text{LDH}}$ 2 Lactate + 2 ß-NAD

Abbreviations used:
ATP = Adenosine 5'-Triphosphate
PRPP = Phosphoribosyl-Pyrophosphate
AMP = Adenosine 5'-Monophosphate
MK = Myokinase
ADP = Adenosine 5'-Diphosphate
PEP = Phospho(enol)pyruvate
PK = Pyruvate Kinase
ß-NADH = ß-Nicotinamide Adenine Dinucleotide, Reduced Form
LDH = Lactic Dehydrogenase
ß-NAD = ß-Nicotinamide Adenine Dinucleotide, Oxidized Form

CONDITIONS: T = 37°C, pH = 7.6, A_{340nm}, Light path = 1 cm

METHOD: Continuous Spectrophotometric Rate Determination

REAGENTS:

A. 125 mM Sodium Phosphate buffer with 7 mM Magnesium Chloride, pH 7.6 at 37°C
(Prepare 100 ml in deionized water using Sodium Phosphate, Monobasic, Anhydrous, Sigma Prod. No. S-0751, and Magnesium Chloride, 4.9 M Solution, Sigma Stock
No. 104-20. Adjust to pH 7.6 at 37°C with 1 M NaOH.)

B. 60 mM Ribose 5-Phosphate Solution (R-5-P)
(Prepare 5 ml in Reagent A using D-Ribose 5-Phosphate, Disodium Salt, Sigma Prod. No. R-7750.)

C. 1.19 M Sodium Bicarbonate Solution
(Prepare 5 ml in deionized water using Sodium Bicarbonate, Sigma Prod. No. S-8875.)

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REAGENTS: (continued)

D. 120 mM Adenosine 5'-Triphosphate Solution (ATP)
(Prepare 1 ml in Reagent C using Adenosine 5'-Triphosphate, Disodium Salt, Sigma Prod. No. A-5394.)

E. 70 mM Phospho(enol)pyruvate Solution (PEP)
(Prepare 1 ml in deionized water using Phospho(enol)pyruvate, Mono(cyclohexylammonium) Salt, Sigma Prod. No. P-3637.)

F. ß-Nicotinamide Adenine Dinucleotide, Reduced Form, (ß-NADH)
(Use one 10 mg vial of ß-Nicotinamide Adenine Dinucleotide, Reduced Form, Disodium Salt, Sigma Stock No. 340-110.)

G. PK/LDH Enzyme Suspension[1] (PK/LDH)
(Use PK/LDH Enzymes suspension, Sigma Stock No. 40-7.)

H. Myokinase Enzyme Suspension[2] (MK)
(Use Myokinase, Sigma Prod. No. M-3382.)

I. Phosphoribosyl-Pyrophosphate Synthetase Enzyme Solution (PRPP Syn)
(Immediately before use, prepare a solution containing 0.1 - 1.0 unit/ml of Phosphoribosyl-Pyrophosphate Synthetase in cold Reagent A. Store on ice.)

PROCEDURE:

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

Reagent D (ATP)	1.00
Reagent E (PEP)	1.00
Reagent A (Buffer)	1.00

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

	Test	Blank
Reaction Cocktail	0.10	0.10
Reagent A (Buffer)	1.00	1.10
Reagent B (R-5-P)	0.10	------
Reagent G (PK/LDH)	0.01	0.01

Reagent H (MK)	0.005	0.005

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PROCEDURE: (continued)

Mix by inversion and equilibrate to 37°C. Then add:

	Test	Blank
Reagent I (PRPP Syn)	0.05	0.05

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

CALCULATIONS:

$$\text{Units/ml enzyme} = \frac{(\Delta A_{340nm}/\text{min Test} - \Delta A_{340nm}/\text{min Blank})(1.265)(df)}{(2)(6.22)(0.05)}$$

1.265 = Total volume (in milliliters) of assay
df = Dilution factor
2 = Conversion factor required since 2 moles of ß-NADH are oxidized per mole of phosphoribosyl-pyrophosphate produced
6.22 = Millimolar extinction coefficient of ß-NADH at 340 nm
0.05 = Volume (in milliliter) of PRPP synthetase used

UNIT DEFINITION:

One unit will catalyze the formation of 1.0 µmole of AMP from ATP and ribose-5-phosphate per minute at pH 7.6 at 37°C.

FINAL ASSAY CONCENTRATIONS:

In a 1.265 ml reaction mix, the final concentrations are 117 mM sodium phosphate, 4.7 mM ribose 5-phosphate, 3.2 mM adenosine 5'-triphosphate, 1.8 mM phospho(enol)pyruvate, 0.34 mM ß-nicotinamide adenine dinucleotide, reduced form, 6.5 mM magnesium chloride, 31 mM sodium bicarbonate, 7 units pyruvate kinase, 10 units lactic dehydrogenase, 10 units myokinase, and 0.005 - 0.05 unit phosphoribosyl-pyrophosphate synthetase.

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REFERENCES:

Remy, C.N., Remy, W.T., and Buchanan, J.M. (1955) *Journal of Biological Chemistry* **217**, 885-895

Kalckar, H.M. (1947) *Journal of Biological Chemistry* **167**, 445-459

NOTES:

1. Contains not less than 700 Pyruvate Kinase units and 1000 L-Lactic Dehydrogenase units per ml.
2. Contains not less than 2000 Myokinase units per ml.
3. Pyruvate Kinase Unit Definition: One unit will convert 1.0 µmole of phos(enol)pyruvate to pyruvate per minute at pH 7.6 at 37°C.
4. L-Lactic Dehydrogenase Unit Definition: One unit will reduce 1.0 µmole of pyruvate to L-lactate per minute at pH 7.5 at 37°C.
5. Myokinase Unit Definition: One unit will convert 2.0 µmoles of ADP to ATP and AMP per minute at pH 7.6 at 37°C.
6. This assay is based on the cited references.
7. Where Sigma Product or Stock numbers are specified, equivalent reagents may be substituted.