Enzymatic Assay of ARGININE KINASE
(EC 2.7.3.3)

PRINCIPLE:

ATP + L-Arginine $\xrightarrow{AK}$ ADP + N$^{?}$-Phospho-L-Arginine

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

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

Abbreviations used:
ATP = Adenosine 5'-Triphosphate
AK = Arginine Kinase
ADP = Adenosine 5'-Diphosphate
PEP = Phospho(enol)pyruvate
PK = Pyruvate Kinase
ß-NADH = ß-Nicotinamide Adenine Dinucleotide, Reduced Form
LDH = L-Lactic Dehydrogenase
ß-NAD = ß-Nicotinamide Adenine Dinucleotide, Oxidized Form

CONDITIONS: T = 30°C, pH = 8.6, A_{340nm}, Light path = 1 cm

METHOD: Continuous Spectrophotometric Rate Determination

REAGENTS:

A. 250 mM Glycine Buffer, pH 8.6 at 30°C
(Prepare 100 ml in deionized water using Glycine, Free Base, Sigma Prod. No. G-7126. Adjust to pH 8.6 at 30°C with 1 M NaOH.)

B. 100 mM Glycine buffer, with 10 mM 2-Mercaptoethanol (Enz Dil)
(Prepare 50 ml in deionized water using Reagent A and 2-Mercaptoethanol, Sigma Prod. No. M-6250.)

C. 200 mM Magnesium Sulfate Solution ($MgSO_4$)
(Prepare 10 ml in deionized water using Magnesium Sulfate, Heptahydrate, Sigma Prod. No. M-1880.)

D. 2 M Potassium Chloride Solution (KCl)
(Prepare 10 ml in deionized water using Potassium Chloride, Sigma Prod. No. P-4504.)

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REAGENTS: (continued)

E. 300 mM Phospho(enol)pyruvate Solution (PEP)
(Prepare 3 ml in deionized water using Phospho(enol)pyruvate, Trisodium Salt, Hydrate, Sigma Prod. No. P-7002.)

F. 200 mM Adenosine 5'-Triphosphate Solution (ATP)
(Prepare 2 ml in deionized water using Adenosine 5'-Triphosphate, Disodium Salt, Sigma Prod. No. A-5394.)

G. 500 mM L-Arginine Solution (ARG)
(Prepare 2 ml in deionized water using L-Arginine, Free Base, Sigma Prod. No. A-5006.)

H. 7.5 mM ß-Nicotinamide Adenine Dinucleotide, Reduced Form, Solution (ß-NADH)
(Dissolve the contents of one 5 mg vial of ß-Nicotinamide Adenine Dinucleotide, Reduced Form, Disodium Salt, Sigma Stock No. 340-105, in the appropriate volume of Reagent A. **PREPARE FRESH**.)

I. PK/LDH Enzyme Solution (PK/LDH)
(Immediately before use, prepare a solution containing 20 units/ml of Pyruvate Kinase in cold Reagent A using PK/LDH Enzymes Suspension, Sigma Stock No. 40-7.[1])

J. Arginine Kinase Enzyme Solution (AK)
(Prepare a solution containing 0.2 - 0.4 unit/ml of Arginine Kinase in Reagent B. Let stand at room temperature for 15 minutes before use to obtain full activation.)

PROCEDURE:

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

Reagent A (Buffer)	19.50
Reagent C ($MgSO_4$)	2.00
Reagent D (KCl)	2.00
Reagent E (PEP)	2.00
Reagent F (ATP)	1.00
Reagent H (ß-NADH)	0.50

Mix by swirling and adjust to pH 8.6 at 30°C with 1 M HCl or 1 M NaOH.

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PROCEDURE: (continued)

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

	Test	Blank
Reaction Cocktail	2.70	2.70
Reagent I (PK/LDH)	0.10	0.10
Reagent G (ARG)	0.10	0.10

Mix by inversion and equilibrate to 30°C. Monitor the A_{340nm} until constant, using a suitably thermostatted spectrophotometer. Then add:

Reagent J (AK)	0.10	------
Reagent B (Enz Dil)	------	0.10

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

CALCULATIONS:

$$\text{Units/ml enzyme} = \frac{(\Delta A_{340nm}/\text{min Test} - \Delta A_{340nm}/\text{min Blank})(3)(df)}{(6.22)(0.1)}$$

3 = Total volume (in milliliters) of assay
df = Dilution factor
6.22 = Millimolar extinction coefficient of ß-NADH at 340 nm
0.1 = Volume (in milliliter) 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 L-arginine and ATP to $N^{?}$-phospho-L-arginine and ADP per minute at pH 8.6 at 30°C.

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FINAL ASSAY CONCENTRATIONS:

In a 3.00 ml reaction mix, the final concentrations are 178 mM glycine, 0.33 mM 2-mercaptoethanol, 13 mM magnesium sulfate, 133 mM potassium chloride, 20 mM phospho(enol) pyruvate, 6.7 mM adenosine 5'-triphosphate, 0.13 mM ß-nicotinamide adenine dinucleotide, reduced form, 2 units pyruvate kinase, 3 units lactic dehydrogenase, 17 mM L-arginine, and 0.02 - 0.04 unit arginine kinase.

REFERENCE:

Blethen, S. (1970) *Methods in Enzymology*, XVIIA, 330-335

NOTES:

1. Contains not less than 700 Pyruvate Kinase units and 1000 L-Lactic Dehydrogenase units per ml.

2. Pyruvate Kinase Unit Definition: One unit will convert 1.0 µmole of phospho(enol)pyruvate to pyruvate per minute at pH 7.6 at 37°C.

3. L-Lactic Dehydrogenase Unit Definition: One unit will reduce 1.0 µmole of pyruvate to L-lactate per minute of 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.