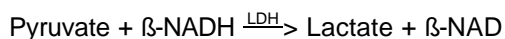
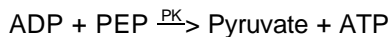


**Enzymatic Assay of ARGININE KINASE  
(EC 2.7.3.3)**

**PRINCIPLE:**



Abbreviations used:

ATP = Adenosine 5'-Triphosphate

AK = Arginine Kinase

ADP = Adenosine 5'-Diphosphate

PEP = Phospho(enol)pyruvate

PK = Pyruvate Kinase

$\beta$ -NADH =  $\beta$ -Nicotinamide Adenine Dinucleotide, Reduced Form

LDH = L-Lactic Dehydrogenase

$\beta$ -NAD =  $\beta$ -Nicotinamide Adenine Dinucleotide, Oxidized Form

**CONDITIONS:** T = 30°C, pH = 8.6,  $A_{340\text{nm}}$ , 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 ( $\text{MgSO}_4$ )  
(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  $\beta$ -Nicotinamide Adenine Dinucleotide, Reduced Form, Solution ( $\beta$ -NADH)  
(Dissolve the contents of one 5 mg vial of  $\beta$ -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.<sup>1</sup>)
- 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 ( $\text{MgSO}_4$ )	2.00
Reagent D (KCl)	2.00
Reagent E (PEP)	2.00
Reagent F (ATP)	1.00
Reagent H ( $\beta$ -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.

	<u>Test</u>	<u>Blank</u>
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  $A_{340\text{nm}}$  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  $A_{340\text{nm}}$  for approximately 5 minutes. Obtain the  $r_{A_{340\text{nm}}}$ /minute using the maximum linear rate for both the Test and Blank.

**CALCULATIONS:**

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

3 = Total volume (in milliliters) of assay

df = Dilution factor

6.22 = Millimolar extinction coefficient of  $\beta$ -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  $\mu\text{mole}$  of L-arginine and ATP to  $N^2$ -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  $\beta$ -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*, XVIIIA, 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  $\mu$ mole 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  $\mu$ mole 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.**