

**Enzymatic Assay of PHOSPHORIBULOKINASE
(EC 2.7.1.19)**

PRINCIPLE:

D-Ribulose 5-Phosphate + ATP $\xrightarrow{\text{PRK}}$ D-Ribulose 1,5-Bisphosphate + ADP

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

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

Abbreviations used:

ATP = Adenosine 5'-Triphosphate

PRK = Phosphoribulokinase

ADP = Adenosine 5'-Diphosphate

PEP = Phospho(enol)pyruvate

PK = Pyruvate Kinase

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

LDH = Lactic Dehydrogenase

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

CONDITIONS: T = 37°C, pH = 7.9, A_{340nm}, Light path = 1 cm

METHOD: Continuous Spectrophotometric Rate Determination

REAGENTS:

- A. 67 mM Tris HCl Buffer, pH 7.9 at 37°C
(Prepare 100 ml in deionized water using Trizma Base, Sigma Prod. No. T-1503. Adjust to pH 7.9 at 37°C with 1 M HCl.)
- B. 50 mM Potassium Chloride Solution (KCl)
(Prepare 10 ml in Reagent A using Potassium Chloride, Sigma Prod. No. P-4504.)
- C. 6 mM Magnesium Sulfate Solution (MgSO₄)
(Prepare 10 ml in Reagent A using Magnesium Sulfate, Heptahydrate, Sigma Prod. No. M-1880.)
- D. 0.54 mM Phospho(enol)pyruvate Solution (PEP)
(Prepare 10 ml in Reagent A using Phospho(enol)pyruvate, Monopotassium Salt, Sigma Prod. No. P-7127.)

**Enzymatic Assay of PHOSPHORIBULOKINASE
(EC 2.7.1.19)**

REAGENTS: (continued)

- E. 0.36 mM Ethylenediaminetetraacetic Acid Solution (EDTA)
(Prepare 10 ml in Reagent A using Ethylenediaminetetraacetic Acid, Disodium Salt, Dihydrate, Sigma Stock No. ED2SS.)
- F. β -Nicotinamide Adenine Dinucleotide, Reduced Form Solution (β -NADH)
(Use β -Nicotinamide Adenine Dinucleotide, Disodium, 1 mg vial, Sigma Stock No. 340-101.)
- G. 50 mM Tris HCl Buffer, pH 7.1 at 37°C (Enz Dil)
(Prepare 25 ml in deionized water using Trizma Base, Sigma Prod. No. T-1503. Adjust to pH 7.1 at 37°C with 1 M HCl.)
- H. 20 mM D-Ribulose 5-Phosphate Solution (RU-5-P)
(Prepare 1 ml in deionized water using D-Ribulose 5-Phosphate, Sodium Salt, Sigma Prod. No. R-9875.)
- I. 9.1 mM Adenosine 5'-Triphosphate Solution (ATP)
(Prepare 1 ml in deionized water using Adenosine 5'-Triphosphate Disodium Salt, Sigma Prod. No. A-5394.)
- J. 82 mM Glutathione, Reduced Form Solution (GSH)
(Prepare 1 ml in deionized water using Glutathione, Reduced Form, Free Acid, Sigma Prod. No. G-4251.)
- K. PK/LDH Enzymes Suspension¹
(Use PK/LDH Enzymes Suspension, Sigma Stock No. 40-7.)
- L. Phosphoribulokinase Enzyme Solution (PRK)
(Immediately before use, prepare a solution containing 0.3 - 0.6 unit/ml of Phosphoribulokinase in cold Reagent G.)

PROCEDURE:

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

Reagent A (Buffer)	60.00
Reagent B (KCl)	10.00
Reagent C (MgSO ₄)	10.00

Reagent D (PEP)	10.00
Reagent E (EDTA)	10.00

**Enzymatic Assay of PHOSPHORIBULOKINASE
(EC 2.7.1.19)**

PROCEDURE: (continued)

Mix by swirling and adjust to pH 7.9 at 37°C with either 1 M HCl or 1 M NaOH, if necessary.

Prepare a final reaction cocktail by pipetting 10 ml of the initial reaction cocktail into Reagent F (β-NADH).

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

	<u>Test</u>	<u>Blank</u>
Final Reaction Cocktail		2.50
		2.50
Reagent I (ATP)	0.10	0.10
Reagent J (GSH)	0.10	0.10
Reagent K (PK/LDH)	0.03	0.03
Reagent L (PRK)	0.10	0.10

Mix by inversion and equilibrate to 37°C. Monitor the $r_{A_{340nm}}$ until constant, using a suitably thermostatted spectrophotometer. Then add:

Reagent H (RU-5-P)	0.10	-----
Deionized Water	-----	0.10

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

CALCULATIONS:

$$\text{Units/ml enzyme} = \frac{(r_{A_{340nm}}/\text{min Test} - r_{A_{340nm}}/\text{Blank})(2.93)(df)}{(6.22)(0.1)}$$

2.93 = Total volume (in milliliters) of assay

df = Dilution factor

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

0.1 = Volume (in milliliter) of enzyme used

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

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

**Enzymatic Assay of PHOSPHORIBULOKINASE
(EC 2.7.1.19)**

UNIT DEFINITION:

One unit will transfer 1.0 μ mole of phosphate from ATP to D-ribulose 5-phosphate per minute at pH 7.9 at 37°C.

FINAL ASSAY CONCENTRATION:

In a 2.93 ml reaction mix, the final concentrations are 57 mM Tris, 4.3 mM potassium chloride, 0.5 mM magnesium sulfate, 0.046 mM phospho(enol)pyruvate, 0.031 mM ethylenediaminetetraacetic acid, 0.31 mM adenosine 5'-triphosphate, 2.8 mM glutathione, reduced form, 0.12 mM β -nicotinamide adenine dinucleotide, reduced form, 0.68 mM D-ribulose 5-phosphate, 21 units pyruvate kinase, 30 units lactic dehydrogenase, and 0.03 - 0.06 unit phosphoribulokinase.

REFERENCE:

Hurwitz, J., Weissbach, A., Horecker, B.L., and Smyrniotis, P.Z. (1956) *Journal of Biological Chemistry* **218**, 769-783

NOTES:

1. Contains not less than 700 Pyruvate Kinase units and 100 Lactic Dehydrogenase units per ml.
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. L-Lactic Dehydrogenase Unit Definition: One unit will reduce 1.0 μ mole of pyruvate to L-lactic 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.