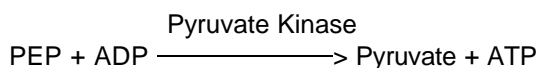
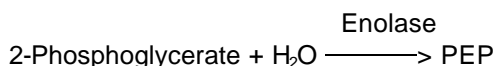


**Enzymatic Assay of ENOLASE  
(EC 4.2.1.11)**

**PRINCIPLE:**



Abbreviations used:

PEP = Phospho(enol)Pyruvate

ADP = Adenosine 5'-Diphosphate

ATP = Adenosine 5'-Triphosphate

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

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

**CONDITIONS:** T = 25°C, pH = 7.4,  $A_{340\text{nm}}$ , Light path = 1 cm

**METHOD:** Continuous Spectrophotometric Rate Determination

**REAGENTS:**

- A. 100 mM Triethanolamine Buffer, pH 7.4 at 25°C  
(Prepare 50 ml in deionized water using Triethanolamine Hydrochloride, Sigma Prod. No. T-1502. Adjust to pH 7.4 at 25°C with 1 M NaOH.)
- B. 56 mM 2-Phosphoglycerate Solution (DPG)  
(Prepare 2 ml in deionized water using D(+)-2-Phosphoglyceric Acid, Sodium Salt, Hydrate, Sigma Prod. No. P-0257.)
- C. 7 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 1 ml of Reagent A or prepare 1 ml in deionized water using  $\beta$ -Nicotinamide Adenine Dinucleotide, Reduced Form, Disodium salt, Sigma Prod. No. N-8129. **PREPARE FRESH.**)
- D. 500 mM Magnesium Sulfate with 2 M Potassium Chloride Solution ( $\text{MgSO}_4/\text{KCl}$ )  
(Prepare 5 ml in deionized water using Magnesium Sulfate, Anhydrous, Sigma Prod. No. M-7506, and Potassium Chloride, Sigma Prod. No. P-4504.)

**Enzymatic Assay of ENOLASE  
(EC 4.2.1.11)**

**REAGENTS:** (continued)

- E. 20 mM Adenosine 5'-Diphosphate Solution (ADP)  
(Prepare 1 ml in deionized water using Adenosine 5'-Diphosphate, Sodium Salt, Sigma Prod. No. A-2754. **PREPARE FRESH.**)
- F. PK/LDH Mixed Enzymes<sup>1</sup>  
(Use PK/LDH Enzyme Solution, Sigma Prod. No. P-0294)
- G. 15 mM Tris HCl with 0.02% (w/v) Bovine Serum Albumin (Enzyme Diluent)  
(Prepare 100 ml in deionized water using Trizma Base, Sigma Prod. No. T-1503, and Albumin, Bovine, A-4503, or equivalent. Adjust to pH 7.4 at 25°C with 1 M HCl.)
- H. Enolase Enzyme Solution  
(Immediately before use, prepare a solution containing 0.25 - 0.5 unit/ml of Enolase in cold Reagent G.)

**PROCEDURE:**

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

	<u>Test</u>	<u>Blank</u>
Reagent A (Buffer)	2.39	2.39
Reagent B (DPG)	0.10	0.10
Reagent C ( $\beta$ -NADH)	0.05	0.05
Reagent D ( $MgSO_4/KCl$ )	0.15	0.15
Reagent E (ADP)	0.20	0.20
Reagent F (PK/LDH)	0.01	0.01

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

Reagent G (Enzyme Diluent)	-----	0.10
Reagent H (Enzyme Solution)	0.10	-----

## Enzymatic Assay of ENOLASE (EC 4.2.1.11)

### PROCEDURE:(continued)

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

### CALCULATIONS:

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

3 = 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 2-phosphoglycerate to phospho(enol)pyruvate per minute at pH 7.4 at 25°C.

### FINAL ASSAY CONCENTRATION:

In a 3.00 ml reaction mix, the final concentrations are 81 mM triethanolamine, 1.9 mM 2-phosphoglycerate, 0.12 mM  $\beta$ -nicotinamide adenine dinucleotide, reduced form, 25 mM magnesium sulfate, 100 mM potassium chloride, 1.3 mM adenosine 5'-diphosphate, 7 units pyruvate kinase, 10 units L-lactic dehydrogenase and 0.025 - 0.05 unit Enolase.

### REFERENCES:

Bergmeyer, H.U. (1974) *Methods in Enzymatic Analysis*, 2nd ed., Vol. II, 449.

### NOTES:

1. Contains approximately 700 units/ml of Pyruvate Kinase and 1000 units/ml of Lactic Dehydrogenase.
2. Pyruvate Kinase unit definition: One unit will convert 1.0  $\mu\text{mole}$  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  $\mu$ mole of pyruvate to L-lactate per minute at pH 7.5 at 37°C.

**Enzymatic Assay of ENOLASE  
(EC 4.2.1.11)**

**NOTES:** (continued)

4. All product and stock numbers, unless otherwise indicated, are Sigma product and stock numbers.

**This procedure is for informational purposes. For a current copy of Sigma's quality control procedure contact our Technical Service Department.**