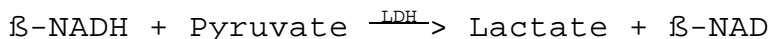


**Determination of the Concentration and  
Molecular Weight of PYRUVIC ACID**

**PRINCIPLE:**



Abbreviations:

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

LDH = Lactic Dehydrogenase

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

**CONDITIONS:** T = 25°C, pH 7.5, A<sub>340nm</sub>, Light path = 1 cm

**METHOD:** Spectrophotometric Determination

**REAGENTS:**

- A. 100 mM Potassium Phosphate Buffer, pH 7.5 at 25°C  
(Prepare 100 ml in deionized water using Potassium Phosphate, Monobasic, Anhydrous, Prod. No. P-5379. Adjust to pH 7.5 with 1 M KOH.)
- B. 0.25 mM Pyruvic Acid Solution (Pyruvate)  
(Immediately before use, prepare 25 ml in cold deionized water.)
- C. 0.30 mM  $\beta$ -Nicotinamide Adenine Dinucleotide, Reduced Form ( $\beta$ -NADH)  
(Prepare 50 ml by dissolving the contents of a 10 mg vial of  $\beta$ -Nicotinamide Adenine Dinucleotide, Reduced Form, Disodium Salt, Sigma Stock No. 340-110 in the appropriate volume of Reagent A. **PREPARE FRESH.** Alternatively, prepare 20 ml in Reagent A using  $\beta$ -Nicotinamide Adenine Dinucleotide, Reduced Form, Disodium Salt, Sigma Prod. No. N-8129.)
- D. Lactic Dehydrogenase Enzyme Solution (LDH)  
(Immediately before use, prepare a solution containing 500 - 1000 units of L-Lactic Dehydrogenase, Sigma Prod. No. L-2500, in cold Reagent A.)

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**PROCEDURE:**

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

	<u>Test</u>	<u>Blank</u>
Reagent B (Pyruvate)	1.00	-----
Deionized Water	-----	1.00
Reagent C (β-NADH)	1.90	1.90

Mix by inversion and equilibrate to 25°C using a suitably thermostatted spectrophotometer. Record the initial  $A_{340\text{nm}}$  for both the Test and Blank. Then add:

Reagent D (LDH)	0.10	0.10
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Immediately mix by inversion and monitor the decrease in  $A_{340\text{nm}}$  until the reaction is complete. Record the final  $A_{340\text{nm}}$  for both the Test and Blank.

**CALCULATIONS:**

$$r A = A_i - A_f$$

$$A_i = \text{Initial Absorbance}$$

$$A_f = \text{Final Absorbance}$$

$$\text{Micromoles Pyruvic acid/ml sample} = \frac{(r A \text{ Test} - r A \text{ Blank})(3)(df)}{(6.22)}$$

3 = Total volume of Reaction Mix

df = Dilution factor

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

$$\text{Apparent molecular weight} = \frac{\mu\text{g sample/mL sample}}{\mu\text{moles of pyruvic acid/ml sample}}$$

1000 = Conversion from mg to μg

$$\% \text{ Purity} = \frac{\text{theoretical molecular weight}}{\text{apparent molecular weight}} \times 100\%$$

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**FINAL ASSAY CONCENTRATION:**

In a 3.00 reaction mix, the final concentrations are 66.7 mM potassium phosphate, 0.19 mM  $\beta$ -nicotinamide adenine dinucleotide, reduced form, 50 - 100 unit L-lactic dehydrogenase, and 0.083 mM pyruvic acid.

**REFERENCE:**

Bergmeyer, H.U. and Bernt, E. (1974) in *Methods of Enzymatic Analysis*, (Bergmeyer, H.U. ed.) Vol. I, 481

**NOTES:**

1. L-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.
2. This procedure is not to be used for the pyruvate impurity in  $\alpha$ -ketoglutaric acid.
3. This assay is based on the cited reference.
4. 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.**