## Determination of the Concentration and Molecular Weight of L-a-GLYCEROPHOSPHATE

**PRINCIPLE:**

L-a-Glycerophosphate + ß-NAD + Hydrazine $\xrightarrow{GDH}$ DAP Hydrazone + NADH

Abbreviations used:
ß-NAD = ß-Nicotinamide Adenine Dinucleotide, Oxidized Form
GDH = a-Glycerophosphate Dehydrogenase
DAP = Dihydroxyacetone Phosphate
ß-NADH = ß-Nicotinamide Adenine Dinucleotide, Reduced Form

**CONDITIONS:** T = 25°C, pH = 9.5, $A_{340nm}$, Light path = 1 cm

**METHOD:** Spectrophotometric Determination

**REAGENTS:**

A. 1 M Glycine Buffer with 609 mM Hydrazine Sulfate and 5.3 mM Ethylenediaminetetraacetic Acid, pH 9.5 at 25°C
(Prepare 50 ml by dissolving Glycine, Free Base, Sigma Prod. No. G-7126, Hydrazine Sulfate, Sigma Prod. No. H-3376, and Ethylenediaminetetraacetic Acid, Tetrasodium Salt, Hydrate, Sigma Stock No. ED4SS, in 2 M NaOH until dissolved. Adjust to pH 9.5 with either 1 M NaOH or 1 M HCl. Then dilute to 50 ml with deionized water.[1] **PREPARE FRESH**.)

B. 20 mM ß-Nicotinamide Adenine Dinucleotide, Oxidized Form, Solution (ß-NAD)
(Prepare by dissolving the contents of one 50 mg vial ß-Nicotinamide Adenine Dinucleotide, Sigma Stock No. 260-150, in the appropriate volume of deionized water. **PREPARE FRESH**.)

C. Glycerophosphate Dehydrogenase Enzyme Solution (GDH)
(Immediately before use, prepare a solution containing 1200 units/ml of a-Glycerophosphate Dehydrogenase, Sigma Prod. No. G-6751, in cold deionized water.)

D. L-a-Glycerophosphate Solution (L-a-GP)
(Immediately before use, prepare a solution containing approximately 0.3 µmoles/ml of L-a-Glycerophosphate in deionized water.)

## Determination of the Concentration and Molecular Weight of L-a-GLYCEROPHOSPHATE

**PROCEDURE:**

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

| | Test | Blank |
|---|---|---|
| Deionized Water | 0.22 | 1.22 |
| Reagent A (Buffer) | 1.35 | 1.35 |
| Reagent D (L-a-GP) | 1.00 | ------ |
| Reagent B (ß-NAD) | 0.38 | 0.38 |

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

| | | |
|---|---|---|
| Reagent C (Enzyme Solution) | 0.05 | 0.05 |

Immediately mix by inversion and allow the reaction to proceed to completion (approximately 5 minutes). Record the final $A_{340nm}$ for both the Test and Blank.

**CALCULATIONS:**

$$r\,A = A_f - A_i$$

$A_f$ = Final Absorbance
$A_i$ = Initial Absorbance

$$\text{Micromoles L-a-GP/ml RM} = \frac{(r\,A_{Test} - r\,A_{Blank})(3)}{(6.22)}$$

L-a-GP = L-a-Glycerophosphate
3 = Total volume (in milliliters) of assay
6.22 = Millimolar extinction coefficient of ß-NADH at 340 nm
RM = Reaction Mixture

$$\text{Apparent Molecular Weight} = \frac{\text{mg/ml L-a-GP} \times 1000}{\text{µmoles of L-a-GP/ml RM}}$$

1000 = Conversion factor from mg to µg
L-a-GP = L-a-Glycerophosphate

## Determination of the Concentration and Molecular Weight of L-a-GLYCEROPHOSPHATE

**FINAL ASSAY CONCENTRATION:**

In a 3.00 ml reaction mix, the final concentrations are 450 mM glycine, 274 mM hydrazine sulfate, 2.4 mM ethylenediaminetetraacetic acid, 2.5 mM ß-nicotinamide adenine dinucleotide, 60 units a-glycerophosphate dehydrogenase, and varying amounts of L-a-glycerophosphate.

**REFERENCE:**

Bergmeyer, H.U., Gawehn, K., and Grassl, M. (1974) in *Methods of Enzymatic Analysis*, (Bergmeyer, H.U. ed) Volume I, 2nd ed., 468-469, Academic Press, New York, NY

**NOTES:**

1. Once the pH of this solution has been adjusted to 9.5 at 25°C, it must be used within two hours. After that period of time, a baseline drift will occur when the solution is measured spectrophotometrically.

2. Where Sigma Product or Stock numbers are specified, equivalent reagents may be substituted.