

Enzymatic Assay of α -GLYCEROPHOSPHATE DEHYDROGENASE (EC 1.1.1.8)

PRINCIPLE:

DHAP + β -NADH $\xrightarrow{\alpha\text{-Glycerophosphate Dehydrogenase}}$ α -Glycerophosphate + β -NAD

Abbreviations used:

DHAP = Dihydroxyacetone Phosphate

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

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

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

METHOD: Continuous Spectrophotometric Rate Determination

REAGENTS:

- A. 300 mM Triethanolamine HCl Buffer, pH 7.4 at 25°C
(Prepare 100 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. 76 mM Dihydroxyacetone Phosphate Solution (DHAP)
(Prepare 1 ml in deionized water using Dihydroxyacetone Phosphate, Lithium Salt, Sigma Prod. No. D-7137.)
- C. 4 mM β -Nicotinamide Adenine Dinucleotide, Reduced Form Solution (β -NADH)
(Prepare 1 ml in Reagent A using β -Nicotinamide Adenine Dinucleotide, Reduced Form, Disodium Salt, Sigma Prod. No. N-8129.)
- D. α -Glycerophosphate Dehydrogenase Enzyme Solution
(Immediately before use, prepare a solution containing 0.15 - 0.30 unit/ml of α -Glycerophosphate Dehydrogenase 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 A (Buffer)	2.70	2.70
Reagent B (DHAP) 0.10	0.10	
Reagent C (β -NADH)	0.10	0.10

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

Reagent D (Enzyme Solution)	0.10	-----
Reagent A (Buffer)	-----	0.10

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

CALCULATIONS:

$$\text{Units/ml enzyme} = \frac{r A_{340\text{nm}}/\text{min Test} - r A_{340\text{nm}}/\text{min Blank})(3)(\text{df})}{(6.22)(0.10)}$$

3 = Volume (in milliliter) of assay

df = Dilution factor

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

0.1 = Volume (in milliliter) of enzyme used

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

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

UNIT DEFINITION:

One unit will convert 1.0 μmole of dihydroxyacetone phosphate to α -glycerophosphate per minute at pH 7.4 at 25°C.

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FINAL ASSAY CONCENTRATION:

In a 3.00 ml reaction mix, the final concentrations are 290 mM triethanolamine, 2.5 mM dihydroxyacetone phosphate, 0.13 mM β -nicotinamide adenine dinucleotide reduced form and 0.015 - 0.03 unit α -glycerophosphate dehydrogenase.

REFERENCES:

Bergmeyer, H.U. (1974) *Methods of Enzymatic Analysis I*, 468.

Beisenherz, G., Bücher, T., and Garbade, K.H. (1955) *Methods in Enzymology I*, 391-397.

NOTES:

1. 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.