

**Enzymatic Assay of GLYOXYLATE REDUCTASE
(EC 1.1.1.26)**

PRINCIPLE:

Glyoxylate + β -NADH $\xrightarrow{\text{Glyoxylate Reductase}}$ Glycolate + β -NAD

Abbreviations used:

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

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

CONDITIONS: T = 25°C, pH = 6.4, A_{340nm}, Light path = 1 cm

METHOD: Continuous Spectrophotometric Rate Determination

REAGENTS:

- A. 50 mM Potassium Phosphate Buffer, pH 6.4 at 25°C
(Prepare 200 ml in deionized water using Potassium Phosphate, Monobasic, Anhydrous, Sigma Prod. No. P-5379. Adjust to pH 6.4 at 25°C with 1 M KOH.)
- B. 702 mM Glyoxylic Acid solution (Glyox)
(Prepare 1 ml in Reagent A using Glyoxylic Acid, Monohydrate, Sodium Salt, Sigma Prod. No. G-4502.)
- C. 12.8 mM β -Nicotinamide Adenine Dinucleotide, Reduced Form Solution (β -NADH)
(Dissolve the contents of one 5 mg vial of β -Nicotinamide Adenine Dinucleotide, Reduced Form, Disodium Salt, Stock No. 340-105, in the appropriate volume of cold Reagent A **or** prepare 1 ml in cold Reagent A using β -Nicotinamide Adenine Dinucleotide, Reduced Form, Disodium Salt, Sigma Prod. No. N-8129. **PREPARE FRESH.**)
- D. Glyoxylate Reductase Enzyme Solution
(Immediately before use, prepare a solution containing 1-2 units/ml of Glyoxylate Reductase in cold deionized water.)

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PROCEDURE:

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

	<u>Test</u>	<u>Blank</u>
Reagent A (Buffer)	2.44	2.44
Reagent B (Glyox)	0.50	0.50
Reagent C (β-NADH)	0.05	0.05

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

Deionized Water	-----	0.10
Reagent D (Enzyme Solution)	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:

$$\text{Units/ml} = \frac{(r A_{340\text{nm}}/\text{min Test} - r A_{340\text{nm}}/\text{min Blank}) (3.09) (df)}{(6.22) (0.1)}$$

3.09 = Total volume (in milliliters) of assay

df = Dilution Factor

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

0.1 = Volume (in milliliters) of enzyme used

RM = Reaction Mix

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

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

UNIT DEFINITION:

One unit will convert 1.0 μmole of glyoxylate to glycolate per minute at pH 6.4 at 25°C.

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FINAL ASSAY CONCENTRATION:

In a 3.09 ml reaction mix, the final concentrations are 48 mM sodium phosphate, 114 mM glyoxylate, 0.2 mM β -nicotinamide adenine dinucleotide, reduced form, and 0.1 - 0.2 unit glyoxylate reductase.

REFERENCE:

Kohn, L.D., Warren, W.A., and Carroll, W.R. (1970). *J. Biol. Chem.* **245**, 3821-3830.

NOTES:

1. This assay is based on the cited reference.
2. 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.