

**Enzymatic Assay of GLUCOSE DEHYDROGENASE
(EC 1.1.1.47)**

PRINCIPLE:

β -D(+) Glucose + β -NAD Glucose Dehydrogenase > D-Glucono-d-Lactone + β -NADH

Abbreviations used:

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

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

CONDITIONS: T = 25°C, pH = 7.6, A_{340nm}, Light path = 1 cm

METHOD: Continuous Spectrophotometric Rate Determination

REAGENTS:

- A. 120 mM Potassium Phosphate Buffer with 150 mM Sodium Chloride, pH 7.6 at 25°C
(Prepare 100 ml in deionized water using Potassium Phosphate, Monobasic, Anhydrous, Prod. No. P-5379, and Sodium Chloride, Prod. No. S-9625. Adjust to pH 7.6 at 25°C with 5 M NaOH.)
- B. 556 mM β -D(+) Glucose Solution (Glucose)
(Prepare 5 ml in Reagent A using β -D(+) Glucose, Prod. No. G-5250.)
- C. 120 mM β -Nicotinamide Adenine Dinucleotide Solution (β -NAD)
(Prepare 1 ml in deionized water using β -Nicotinamide Adenine Dinucleotide, Prod. No. N-7004 or dissolve the contents of one 50 mg vial of β -Nicotinamide Adenine Dinucleotide, Stock No. 260-150, in the appropriate volume of deionized water. **PREPARE FRESH.**)
- D. Glucose Dehydrogenase Enzyme Solution
(Immediately before use, prepare a solution containing 0.3 - 0.6 unit/ml of Glucose 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)	2.00	2.10
Reagent B (Glucose)	1.00	1.00
Reagent D (Enzyme Solution)	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 C (β -NAD)	0.05	0.05
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Immediately mix by inversion and record the increase in $A_{340\text{nm}}$ for approximately 20 minutes. Obtain the $r A_{340\text{nm}}/\text{minute}$ using the maximum linear rate for both the Test and Blank.

CALCULATIONS:

$$\text{Units/ml enzyme} = \frac{(r A_{340\text{nm}}/\text{min Test} - r A_{340\text{nm}}/\text{min Blank})(3.15)(\text{df})}{(6.22)(0.1)}$$

3.15 = Total volume (in milliliter) of enzyme 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 oxidize 1.0 μmole of β -D-glucose to D-glucono-D-lactone per minute at pH 7.6 at 25°C.

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FINAL ASSAY CONCENTRATION:

In a 3.15 ml reaction mix, the final concentrations are 118 mM potassium phosphate, 148 mM sodium chloride, 177 mM glucose, 2 mM β -nicotinamide adenine dinucleotide, and 0.03 - 0.06 unit glucose dehydrogenase.

NOTES:

1. 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.