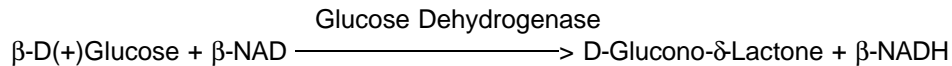


Enzymatic Assay of GLUCOSE DEHYDROGENASE (EC 1.1.1.47)

PRINCIPLE:



Abbreviations used:

$\beta\text{-NAD}$ = β -Nicotinamide Adenine Dinucleotide, Oxidized Form

$\beta\text{-NADH}$ = β -Nicotinamide Adenine Dinucleotide, Reduced Form

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

METHOD: Continuous Spectrophotometric Rate Determination

REAGENTS:

- A. 100 mM Potassium Phosphate Buffer, pH 7.6 at 25°C
(Prepare 100 ml in deionized water using Potassium Phosphate, Monobasic, Anhydrous, Sigma Prod. No. P-5379. Adjust to pH 7.6 at 25°C with 5 M KOH.)
- B. 1000 mM $\beta\text{-D}(+)\text{Glucose}$ Solution (Glucose)
(Prepare 10 ml in deionized water using $\beta\text{-D}(+)\text{Glucose}$, Sigma Prod. No. G-5250.¹)
- C. 20 mM β -Nicotinamide Adenine Dinucleotide Solution ($\beta\text{-NAD}$)
(Prepare 1 ml in deionized water using β -Nicotinamide Adenine Dinucleotide, Sigma Prod. No. N-7004 or dissolve the contents of one 20 mg vial of β -Nicotinamide Adenine Dinucleotide, Sigma Stock No. 260-120, 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 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)	1.80	1.80
Reagent B (Glucose)	1.00	1.00
Reagent C (β-NAD) 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	-----
Deionized Water	-----	0.10

Immediately mix by inversion and record the increase in $A_{340\text{nm}}$ for approximately 10 minutes. Obtain the $\Delta A_{340\text{nm}}/\text{minute}$ using the maximum linear rate for both the Test and Blank.

CALCULATIONS:

$$\text{Units/mg enzyme} = \frac{\Delta A_{340\text{nm}}/\text{min Test} - \Delta A_{340\text{nm}}/\text{min Blank}}{(6.22) (\text{mg enzyme/ml RM})}$$

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

UNIT DEFINITION:

One unit will oxidize 1.0 μmole of β-D-glucose to D-glucono-δ-lactone per minute at pH 7.6 at 25°C.

FINAL ASSAY CONCENTRATION:

In a 3.00 ml reaction mix, the final concentrations are 60 mM potassium phosphate, 333 mM glucose, 0.67 mM β-NAD and 0.03 - 0.06 unit glucose dehydrogenase.

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(EC 1.1.1.47)**

REFERENCES:

Strecker, H.J. (1955) *Methods in Enzymology* **1**, 335.

NOTES:

1. This enzyme is only to be assayed using β -D(+)-glucose. It is highly specific for the β -form.
2. 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.