Enzymatic Assay of GLUCOSE DEHYDROGENASE
(EC 1.1.1.47)

PRINCIPLE:

$$\beta\text{-D(+)Glucose} + \beta\text{-NAD} \xrightarrow{\text{Glucose Dehydrogenase}} \text{D-Glucono-}\delta\text{-Lactone} + \beta\text{-NADH}$$

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. 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 β-D(+)Glucose Solution (Glucose)
(Prepare 10 ml in deionized water using β-D(+) Glucose, Sigma Prod. No. G-5250.[1])

C. 20 mM β-Nicotinamide Adenine Dinucleotide Solution (β-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:

		Test	Blank
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_{340nm} 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_{340nm} for approximately 10 minutes. Obtain the ΔA_{340nm}/minute using the maximum linear rate for both the Test and Blank.

CALCULATIONS:

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

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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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.