Enzymatic Assay of GLUCOSE DEHYDROGENASE
(EC 1.1.1.47)

PRINCIPLE:

ß-D-(+)-Glucose + ß-NADP $\xrightarrow{\text{Glucose Dehydrogenase}}$ D-Glucono-1,4-Lactone + ß-NADPH

Abbreviations used:
ß-NADP = ß-Nicotinamide Adenine Dinucleotide Phosphate, Oxidized Form
ß-NADPH = ß-Nicotinamide Adenine Dinucleotide Phosphate, Reduced Form

CONDITIONS: T = 37°C, pH 7.0, A_{340nm}, Light path = 1 cm

METHOD: Continuous Spectrophotometric Rate Determination

REAGENTS:

A. 50 mM Sodium Phosphate Buffer, pH 7.0 at 37°C
(Prepare 100 ml in deionized water using Sodium Phosphate, Monobasic, Monohydrate, Sigma Prod. No. S-9638. Adjust to pH 7.0 at 37°C with 1 M NaOH.)

B. 500 mM ß-$_D$(+)Glucose Solution (Glucose)
(Prepare 10 ml in deionized water using ß-$_D$(+)Glucose, Sigma Prod. No. G-5250. **PREPARE FRESH**.)

C. 4.0 mM ß-Nicotinamide Adenine Dinucleotide, Phosphate, Oxidized Form Solution (ß-NADP)
(Prepare by dissolving the contents of one 10 mg vial of ß-Nicotinamide Adenine Dinucleotide Phosphate, Sodium, Sigma Stock No. 240-310, 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:

	Test	Blank
Reagent A (Buffer)	0.70	0.80
Reagent B (Glucose)	0.10	0.10
Reagent C (ß-NADP)	0.10	0.10

Mix by inversion and equilibrate to 37°C. Monitor the A_{340nm} until constant, using a suitably thermostatted spectrophotometer. Then add:

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

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

CALCULATIONS:

$$\text{Units/ml enzyme} = \frac{(r\ A_{340nm}/\text{min Test} - r\ A_{340nm}/\text{min Blank})(1)(df)}{(6.22)(0.1)}$$

1 = Volume (in milliliter) of assay
df = Dilution factor
6.22 = Millimolar extinction coefficient of ß-NADPH at 340nm
0.1 = Volume (in milliliter) of enzyme used

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

UNIT DEFINITION:

One unit will oxidize 1.0 µmole per minute of ß-D-glucose to D-glucono-?-lactone or D-galactose to D-galactonate at pH 7.0 at 37°C in the presence of $NADP^+$.

FINAL ASSAY CONCENTRATIONS:

In a 1.00 ml reaction mix, the final concentrations are 40 mM sodium phosphate, 50 mM ß-D(+)glucose, 0.4 mM ß-nicotinamide adenine dinucleotide phosphate, and 0.03 - 0.06 unit glucose dehydrogenase.

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REFERENCE:

Smith, L.D., Budgen, N., Bungard, S.J., Danson, M.J., and Hough, D.W., (1989) *Biochemical Journal* **261**, 973-977

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.