Enzymatic Assay of GLUCOSE DEHYDROGENASE
(EC 1.1.1.47)

**PRINCIPLE:**

\[
\beta-D-(+)-\text{Glucose} + \beta-\text{NADP} \xrightarrow{\text{Glucose Dehydrogenase}} \text{D-Glucono-1,4-Lactone} + \beta-\text{NADPH}
\]

Abbreviations used:
- \(\beta-\text{NADP}\) = \(\beta\)-Nicotinamide Adenine Dinucleotide Phosphate, Oxidized Form
- \(\beta-\text{NADPH}\) = \(\beta\)-Nicotinamide Adenine Dinucleotide Phosphate, Reduced Form

**CONDITIONS:**  \(T = 37^\circ\text{C}, \ \text{pH 7.0}, \ A_{340\text{nm}}, \ \text{Light path} = 1 \ \text{cm}\)

**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. Adjust to pH 7.0 at 37°C with 1 M NaOH.)

B. 500 mM \(\beta-D-(+)-\text{Glucose}\) Solution (Glucose) (Prepare 10 ml in deionized water using \(\beta-D-(+)-\text{Glucose}\), PREPARE FRESH.)

C. 4.0 mM \(\beta\)-Nicotinamide Adenine Dinucleotide, Phosphate, Oxidized Form Solution (\(\beta-\text{NADP}\)) (Prepare by dissolving the contents of one 10 mg vial of \(\beta\)-Nicotinamide Adenine Dinucleotide Phosphate, Sodium, 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.)
Enzymatic Assay of GLUCOSE DEHYDROGENASE (EC 1.1.1.47)

PROCEDURE:

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

<table>
<thead>
<tr>
<th></th>
<th>Test</th>
<th>Blank</th>
</tr>
</thead>
<tbody>
<tr>
<td>Reagent A (Buffer)</td>
<td>0.70</td>
<td>0.80</td>
</tr>
<tr>
<td>Reagent B (Glucose)</td>
<td>0.10</td>
<td>0.10</td>
</tr>
<tr>
<td>Reagent C (β-NADP)</td>
<td>0.10</td>
<td>0.10</td>
</tr>
</tbody>
</table>

Mix by inversion and equilibrate to 37°C. Monitor the A$_{340\text{nm}}$ 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$_{340\text{nm}}$ for approximately 5 minutes. Obtain the $r_{A_{340\text{nm}}/\text{min}}$ using the maximum linear rate for both the Test and Blank.

CALCULATIONS:

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

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

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 β-D-glucose to D-glucono-δ-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 β-D(+)glucose, 0.4 mM β-nicotinamide adenine dinucleotide phosphate, and 0.03 - 0.06 unit glucose dehydrogenase.