Enzymatic Assay of GLUCOKINASE  
(EC 2.7.1.2)

**PRINCIPLE:**

\[ \beta-D(+)\text{Glucose} + \text{ATP} \xrightarrow{\text{Glucokinase}} \text{d-Glucose 6-Phosphate} + \text{ADP} \]

\[ \text{d-Glucose 6-Phosphate} + \beta-\text{NADP} \xrightarrow{G-6PDH} \text{6-Phospho-}D\text{-gluconate} + \beta-\text{NADPH} \]

**Abbreviations:**

ATP = Adenosine 5'-Triphosphate
ADP = Adenosine 5'-Diphosphate
β-NADP = β-Nicotinamide Adenine Dinucleotide Phosphate, Oxidized Form
β-NADPH = β-Nicotinamide Adenine Dinucleotide Phosphate, Reduced Form
G-6PDH = Glucose-6-Phosphate Dehydrogenase

**CONDITIONS:**  \( T = 30^\circ C, \text{pH} = 9.0, A_{340nm}, \text{Light path} = 1 \text{ cm} \)

**METHOD:** Continuous Spectrophotometric Rate Determination

**REAGENTS:**

A. 75 mM Tris HCl Buffer, pH 9.0 at 30°C
   (Prepare 100 ml in deionized water using Trizma Base, Adjust to pH 9.0 at 30°C with 1 M HCl.)

B. 600 mM Magnesium Chloride Solution (MgCl₂)
   (Prepare 10 ml in deionized water using Magnesium Chloride, Hexahydrate.)

C. 120 mM Adenosine Triphosphate Solution (ATP)
   (Prepare 10 ml in deionized water using Adenosine 5'-Triphosphate, Disodium Salt.)

D. 360 mM β-D(+)Glucose Solution (Glucose)
   (Prepare 10 ml in deionized water using β-D(+)Glucose.)
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PROCEDURE: (continued)

E. 27 mM β-Nicotinamide Adenine Dinucleotide Phosphate,  
Oxidized Form Solution (β-NADP)  
(Dissolve the contents of one 30 mg vial of  
β-Nicotinamide Adenine Dinucleotide Phosphate, Sodium  
Salt, in the appropriate  
volume of deionized water.)

F. Glucose-6-Phosphate Dehydrogenase Enzyme Solution  
(G-6PDH)  
(Immediately before use, prepare a solution containing  
100 units/ml of Glucose-6-Phosphate Dehydrogenase,  
in cold deionized water.)

G. 50 mM Tris HCl Buffer, pH 8.5 at 30°C (Enzyme Diluent)  
(Prepare 50 ml in deionized water using Trizma Base,  
Adjust to pH 8.5 at 30°C with  
1 M HCl.)

H. Glucokinase Enzyme Solution (GLCK)  
(Immediately before use, prepare a solution containing  
0.25 - 0.50 unit/ml of Glucokinase in cold Reagent G.)

PROCEDURE:

Prepare a reaction cocktail by pipetting (in milliliters)  
the following reagents into a suitable container:

<table>
<thead>
<tr>
<th>Reagent</th>
<th>Volume (ml)</th>
</tr>
</thead>
<tbody>
<tr>
<td>A (Buffer)</td>
<td>24.00</td>
</tr>
<tr>
<td>B (MgCl₂)</td>
<td>1.00</td>
</tr>
<tr>
<td>C (ATP)</td>
<td>1.00</td>
</tr>
<tr>
<td>D (Glucose)</td>
<td>1.00</td>
</tr>
<tr>
<td>E (β-NADP)</td>
<td>1.00</td>
</tr>
</tbody>
</table>

Mix by swirling and adjust to pH 9.0 at 30°C with 1 M HCl  
or 1 M NaOH, if necessary.

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

<table>
<thead>
<tr>
<th>Reagent</th>
<th>Test</th>
<th>Blank</th>
</tr>
</thead>
<tbody>
<tr>
<td>Reaction Cocktail</td>
<td>2.80</td>
<td>2.80</td>
</tr>
<tr>
<td>Reagent F (6-GPDH)</td>
<td>0.10</td>
<td>0.10</td>
</tr>
</tbody>
</table>
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PROCEDURE:  (continued)

Mix by inversion and equilibrate to 30°C. Monitor the
A$_{340nm}$ until constant using a suitably thermostatted
spectrophotometer. Then add:

<table>
<thead>
<tr>
<th>Reagent H (GLCK)</th>
<th>0.10</th>
<th>------</th>
</tr>
</thead>
<tbody>
<tr>
<td>Reagent G (Enzyme Diluent)</td>
<td>------</td>
<td>0.10</td>
</tr>
</tbody>
</table>

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

CALCULATION:

$\text{Units/ml enzyme} = \frac{(r_{\text{A}_{340nm}/\text{min Test}} - r_{\text{A}_{340nm}/\text{min Blank}})(3)}{(6.22)(0.1)}$

3 = Volume (in milliliters) of assay
6.22 = Millimolar extinction coefficient of ß-NADPH at 340
nm
0.1 = Volume (in milliliters) of enzyme used

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

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

UNIT DEFINITION:

One unit will phosphorylate 1.0 µmole of D-glucose to
D-glucose 6-phosphate per minute at pH 9.0 at 30°C.

FINAL ASSAY CONCENTRATIONS:

In a 3.00 ml reaction mix, the final concentrations are
60 mM Tris, 20 mM magnesium chloride, 4.0 mM adenosine
5'-triphosphate, 12.0 mM glucose, 0.9 mM ß-nicotinamide adenine dinucleotide phosphate, 10 units glucose 6-
phosphate dehydrogenase and 0.025 - 0.050 unit
glucokinase.

REFERENCE:

420.
NOTES:

1. Glucose-6-Phosphate Dehydrogenase Unit Definition: One unit will oxidize 1.0 μmole of D-glucose 6-phosphate to 6-phospho-D-gluconate per minute in the presence of NADP at pH 7.4 at 25°C.

2. This assay is based on the cited reference.