

**Enzymatic Assay of GLUCOKINASE
(EC 2.7.1.2)**

PRINCIPLE:

β -D(+)-Glucose + ATP $\xrightarrow{\text{Glucokinase}}$ D-Glucose 6-Phosphate + ADP

D-Glucose 6-Phosphate + β -NADP $\xrightarrow{\text{G-6PDH}}$ 6-Phospho-D-gluconate + β -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°C, pH = 9.0, A_{340nm}, Light path = 1 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, Sigma Prod. No. T-1503. 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, Sigma Prod. No. M-0250.)
- C. 120 mM Adenosine Triphosphate Solution (ATP)
(Prepare 10 ml in deionized water using Adenosine 5'-Triphosphate, Disodium Salt, Sigma Prod. No. A-5394.)
- D. 360 mM β -D(+)-Glucose Solution (Glucose)
(Prepare 10 ml in deionized water using β -D(+)-Glucose, Sigma Prod. No. G-5250.)

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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, Sigma Stock No. 240-330 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, Sigma Prod. No. G-6378 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, Sigma Prod. No. T-1503. 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:

Reagent A (Buffer)	24.00
Reagent B ($MgCl_2$)	1.00
Reagent C (ATP)	1.00
Reagent D (Glucose)	1.00
Reagent E (β -NADP)	1.00

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:

	<u>Test</u>	<u>Blank</u>
Reaction Cocktail	2.80	2.80
Reagent F (6-GPDH)	0.10	0.10

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PROCEDURE: (continued)

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

Reagent H (GLCK)	0.10	-----
Reagent G (Enzyme Diluent)	-----	0.10

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

CALCULATION:

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

3 = Volume (in milliliters) of assay

6.22 = Millimolar extinction coefficient of β -NADPH at 340 nm

0.1 = Volume (in milliliters) of enzyme used

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

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

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 β -nicotinamide adenine dinucleotide phosphate, 10 units glucose 6-phosphate dehydrogenase and 0.025 - 0.050 unit glucokinase.

REFERENCE:

Goward, C.R., *et al* (1986) *Biochemical Journal* **237**, 415-420.

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NOTES:

1. Glucose-6-Phosphate Dehydrogenase Unit Definition:
One unit will oxidize 1.0 μ mole 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.
3. 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.