SIGMA QUALITY CONTROL TEST PROCEDURE

ProductInformation

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 ($MgCl_2$)
(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.)

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:

	Test	Blank
Reaction Cocktail	2.80	2.80
Reagent F (6-GPDH)	0.10	0.10

PROCEDURE: (continued)

Mix by inversion and equilibrate to 30°C. Monitor the A_{340nm} 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_{340nm} for approximately 5 minutes. Obtain the ΔA_{340nm}/minute using the maximum linear rate for both the Test and Blank.

CALCULATION:

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

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 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.

3. Where Sigma Product or Stock numbers are specified, equivalent reagents may be substituted.