SIGMA QUALITY CONTROL TEST PROCEDURE

ProductInformation

Enzymatic Assay of HEXOKINASE[1]
(EC 2.7.1.1)

PRINCIPLE:

D-Glucose + ATP $\xrightarrow{\text{Hexokinase}}$ D-Glucose 6-Phosphate + ADP

D-Glucose 6-Phosphate + β-NADP $\xrightarrow{\text{G-6-PDH}}$ 6-PG + β-NADPH

Abbreviations used:
ATP = Adenosine 5'-Triphosphate
ADP = Adenosine 5'-Diphosphate
G-6-PDH = Glucose-6-Phosphate Dehydrogenase
β-NADP = β-Nicotinamide Adenine Dinucleotide Phosphate, Oxidized Form
β-NADPH = β-Nicotinamide Adenine Dinucleotide Phosphate, Reduced Form
6-PG = 6-Phospho-D-Gluconate

CONDITIONS: T = 25°C, pH = 7.6, A_{340nm}, Light path = 1 cm

METHOD: Continuous Spectrophotometric Rate Determination

REAGENTS:

A. 50 mM Triethanolamine Buffer, pH 7.6 at 25°C
(Prepare 100 ml in deionized water using Triethanolamine Hydrochloride, Sigma Prod. No. T-1502. Adjust to pH 7.6 at 25°C with 1 M NaOH.)

B. 555 mM D-Glucose Solution
(Prepare 10 ml in Reagent A using D-(+)-Glucose, Anhydrous, Sigma Prod. No. G-8270.)

C. 19 mM Adenosine 5'-Triphosphate Solution (ATP)
(Prepare 10 ml in deionized water using Adenosine 5'-Triphosphate, Disodium Salt, Sigma Prod. No. A-2383. **PREPARE FRESH.**)

D. 100 mM Magnesium Chloride Solution ($MgCl_2$)
(Prepare 5 ml in deionized water using Magnesium Chloride, Hexahydrate, Sigma Prod. No. M-0250.)

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REAGENTS: (continued)

E. 14 mM β-Nicotinamide Adenine Dinucleotide Phosphate, Oxidized Form, Solution (β-NADP)
(Dissolve the contents of two 10 mg vials of β-Nicotinamide Adenine Dinucleotide Phosphate, Sodium Salt, Sigma Stock No. 240-310, in the appropriate volume of deionized water **or** prepare 10 ml in deionized water using β-Nicotinamide Adenine Dinucleotide Phosphate, Sodium Salt, Sigma Prod. No. N-0505. **PREPARE FRESH**.)

F. Glucose-6-Phosphate Dehydrogenase Enzyme Solution (G-6-PDH)[2]
(Immediately before use, prepare a solution containing 125 units/ml of Glucose-6-Phosphate Dehydrogenase, Sigma Prod. No. G-4134, in cold Reagent A.)[3]

G. Hexokinase Enzyme Solution
(Immediately before use, prepare a solution containing 0.5 - 1.0 unit/ml of Hexokinase in cold deionized water.)

PROCEDURE:

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

	Test	Blank
Reagent A (Buffer)	1.00	1.00
Reagent B (D-Glucose)	1.00	1.00
Reagent C (ATP)	0.10	0.10
Reagent D ($MgCl_2$)	0.20	0.20
Reagent E (β-NADP)	0.20	0.20
Reagent F (G-6-PDH)	0.02	0.02

Mix by inversion and equilibrate to 25°C. Monitor the A_{340nm} until constant, using a suitably thermostatted spectrophotometer. Then add:

Deionized Water	------	0.05
Reagent G (Enzyme Solution)	0.05	------

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.

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CALCULATIONS:

$$\text{Units/ml enzyme} = \frac{(\Delta A_{340nm}/\text{min Test} - \Delta A_{340nm}/\text{min Blank})(2.57)(df)}{(6.22)(0.05)}$$

2.57 = Total volume (in milliliters) of assay
df = Dilution factor
6.22 = Millimolar extinction coefficient of β-NADPH at 340 nm
0.05 = Volume (in milliliter) 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 per minute at pH 7.6 at 25°C.

FINAL ASSAY CONCENTRATION:

In a 2.57 ml reaction mix, the final concentrations are 39 mM triethanolamine, 216 mM D-glucose, 0.74 mM adenosine 5'-triphosphate, 7.8 mM magnesium chloride, 1.1 mM β-nicotinamide adenine dinucleotide phosphate, 2.5 units glucose-6-phosphate dehydrogenase, and 0.025 - 0.05 unit of hexokinase.

REFERENCES:

Bergmeyer, H.U., Grassl, M., and Walter, H.E. (1983) in *Methods of Enzymatic Analysis* (Bergmeyer, H.U. ed) 3rd ed., Volume II, 222-223, Verlag Chemie, Deerfield Beach, FL

NOTES:

1. This procedure is not to be used to assay the activity of Hexokinase, Sigma Prod. No. H-3779, Hexokinase, Insoluble enzyme attached to beaded agarose, Sigma Prod. No. H-2005, and Hexokinase, Insoluble enzyme attached to polyacrylamide, Sigma Prod. No. H-8254.

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NOTES

2. 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 β-NADP at pH 7.4 at 25°C.

3. Other types of glucose-6-phosphate dehydrogenase may contain varying amounts of hexokinase as an impurity.

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