

**Enzymatic Assay of HEXOKINASE
(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 = 30°C, pH = 7.6, A_{340nm}, Light path = 1 cm

METHOD: Continuous Spectrophotometric Rate Determination

REAGENTS:

- A. 50 mM Tris HCl Buffer, pH 7.6 at 30°C
(Prepare 500 ml in deionized water using Trizma Hydrochloride, Sigma Prod. No. T-3253. Adjust to pH 7.6 at 30°C with 1 M NaOH.)
- B. 50 mM D-Glucose Solution (D-Glucose)
(Prepare 10 ml in Reagent A using D-(+)-Glucose, Anhydrous, Mixed Anomers, Sigma Prod. No. G-8270.)
- C. 30 mM Adenosine 5'-Triphosphate Solution (ATP)
(Prepare 10 ml in Reagent A using Adenosine 5'-Triphosphate, Disodium Salt, Sigma Prod. No. A-2383. **PREPARE FRESH.**)
- D. 200 mM Magnesium Chloride Solution (MgCl₂)
(Prepare 5 ml in Reagent A using Magnesium Chloride, Hexahydrate, Sigma Prod. No. M-0250.)

**Enzymatic Assay of HEXOKINASE
(EC 2.7.1.1)**

REAGENTS: (continued)

- E. 1 mM β -Nicotinamide Adenine Dinucleotide Phosphate Solution (β -NADP)
(Dissolve the contents of one 10 mg vial of β -Nicotinamide Adenine Dinucleotide Phosphate, Sodium Salt, Sigma Stock No. 240-310, in the appropriate volume of Reagent A. **PREPARE FRESH.**)
- F. Glucose-6-Phosphate Dehydrogenase Enzyme Solution (G-6-PDH)
(Immediately before use, prepare a solution containing 500 units/ml in cold Reagent A using Glucose-6-Phosphate Dehydrogenase, Sigma Prod. No. G-5885.)
- G. 100 mM D-Glucose Solution (Enzyme Diluent)
(Prepare 50 ml in Reagent A using D-(+)-Glucose, Anhydrous, Mixed Anomers, Sigma Prod. No. G-8270.)
- H. Hexokinase Enzyme Solution (HK)
(Immediately before use, prepare a solution containing 0.25 unit/ml of Hexokinase in cold Reagent G.)

PROCEDURE:

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

Reagent A (Buffer)	11.00
Reagent B (D-Glucose)	3.00
Reagent C (ATP)	10.00
Reagent D (MgCl ₂)	3.00

Mix and adjust to pH 7.6 at 30°C with 0.1 M HCl or 0.1 M NaOH, if necessary.

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

	<u>Test</u>	<u>Blank</u>
Reaction Cocktail	2.70	2.70
Reagent E (β -NADP)	0.30	0.30
Reagent F (G-6-PDH)	0.01	0.01

**Enzymatic Assay of HEXOKINASE
(EC 2.7.1.1)**

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:

	<u>Test</u>	<u>Blank</u>
Reagent G (Enzyme Diluent)	-----	0.10
Reagent H (Enzyme Solution)	0.10	-----

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

CALCULATIONS:

$$\text{Units/ml enzyme} = \frac{(r A_{340\text{nm}}/\text{min Test} - r A_{340\text{nm}}/\text{min Blank})(3.11)(\text{df})}{(6.22)(0.1)}$$

3.11 = Total volume (in milliliters) of assay

df = Dilution factor

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 μ mole of D-glucose per minute at pH 7.6 at 30°C.

FINAL ASSAY CONCENTRATION:

In a 3.11 ml reaction mix, the final concentrations are 50 mM Tris, 8.0 mM D-glucose, 9.6 mM adenosine 5'-triphosphate, 19 mM magnesium chloride, 0.1 mM β -nicotinamide adenine dinucleotide phosphate, 5.0 units glucose-6-phosphate dehydrogenase and 0.025 unit hexokinase.

**Enzymatic Assay of HEXOKINASE
(EC 2.7.1.1)**

REFERENCE:

Easterby, J.S., O'Brien, M.J. (1973) *Eur. J. Biochem.* **38**, 201-211

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 β -NADP at pH 7.8 at 30°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.