上海源叶生物科技有限公司
Shanghai yuanye Bio-Technology Co., Ltd
电话：021-61312973　传真：021-55068248
网址：www.shyuanye.com
邮箱：shyysw@sina.com

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, 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)

C. 19 mM Adenosine 5'-Triphosphate Solution (ATP)
(Prepare 10 ml in deionized water using Adenosine 5'-Triphosphate, Disodium Salt, **PREPARE FRESH.**)

D. 100 mM Magnesium Chloride Solution ($MgCl_2$)
(Prepare 5 ml in deionized water using Magnesium Chloride, Hexahydrate,)

Enzymatic Assay of HEXOKINASE[1]
(EC 2.7.1.1)

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. **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, 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.

上海源叶生物科技有限公司
Shanghai yuanye Bio-Technology Co., Ltd
电话：021-61312973　传真：021-55068248
网址：www.shyuanye.com
邮箱：shyysw@sina.com

Enzymatic Assay of HEXOKINASE[1]
(EC 2.7.1.1)

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,Hexokinase, Insoluble enzyme attached to beaded agarose, and Hexokinase, Insoluble enzyme attached to polyacrylamide

Enzymatic Assay of HEXOKINASE[1]
(EC 2.7.1.1)

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 Yuanye Product or Stock numbers are specified, equivalent reagents may be substituted.

Yuanye warrants that the above procedure information is currently utilized at Yuanye and that Yuanye products conform to the information in Yuanye publications. Purchaser must determine the suitability of the information and products for its particular use. Upon purchase of products, see reverse side of invoice or packing slip for additional terms and conditions of sale.