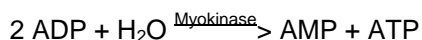


Product Information

SIGMA QUALITY CONTROL TEST PROCEDURE

Enzymatic Assay of MYOKINASE (EC 2.7.4.3)

PRINCIPLE:



Abbreviations:

ADP = Adenosine 5'-Diphosphate

AMP = Adenosine 5'-Monophosphate

ATP = Adenosine 5'-Triphosphate

G-6-P = Glucose 6'-Phosphate

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

CONDITIONS: T= 37°C, pH 7.6, A_{340nm}, Light path = 1 cm

METHOD: Continuous Spectrophotometric Rate Determination

REAGENTS:

- A. 250 mM Glycylglycine Buffer, pH 7.6 at 37°C
(Prepare 100 ml in deionized water using Gly-Gly, Free Base, Prod. No. G-1002. Adjust to pH 7.6 at 37°C with 1 M NaOH.)
- B. 40 mM Adenosine Diphosphate Solution (ADP)
(Prepare 2 ml in deionized water using Adenosine 5'-Diphosphate, Sodium Salt, Prod. No. A-2754.)
- C. 20 mM β -Nicotinamide Adenine Dinucleotide Phosphate, Oxidized Form Solution (β -NADP)
(Prepare 5 ml in deionized water using β -Nicotinamide Adenine Dinucleotide Phosphate, Sodium Salt, Prod. No. N-0505. **PREPARE FRESH.**)
- D. 300 mM Magnesium Chloride Solution (MgCl₂)
(Prepare 2.4 ml in deionized water using Magnesium Chloride, Hexahydrate, Prod. No. M-0250.)
- E. 1000 mM β -D(+)-Glucose Solution (Glucose)
(Prepare 1 ml in deionized water using β -D(+)-Glucose, Prod. No. G-5250.)
- F. Hexokinase and Glucose-6-Phosphate Dehydrogenase Solution (G-6-PDH/Hex)

(Immediately before use, prepare a solution containing 10 units/ml of Glucose-6-Phosphate Dehydrogenase activity and 20 units/ml of Hexokinase activity in cold deionized water using Hexokinase and Glucose-6-Phosphate Dehydrogenase (G-6-PDH), Prod. No. H-8629.)

Reagents (continued)

- G. 0.1% (w/v) Bovine Serum Albumin Solution (BSA)
(Prepare 100 ml in Reagent A using Albumin, Bovine, Prod. No. A-4503 or equivalent.)
- H. Myokinase Enzyme Solution (Myokinase)
(Immediately before use, prepare a solution containing 0.2 - 0.6 unit/ml of Myokinase in cold Reagent G.)

PROCEDURE:

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

	<u>Test</u>	<u>Blank</u>
Deionized Water	1.57	1.57
Reagent A (Buffer)	0.60	0.60
Reagent B (ADP)	0.15	0.15
Reagent C (β-NADP)	0.35	0.35
Reagent D (MgCl ₂)	0.10	0.10
Reagent E (Glucose)	0.03	0.03
Reagent F (G-6-PDH/Hex)	0.10	0.10

Mix by inversion and equilibrate to 37°C. Monitor the A_{340nm} until constant, using a suitably thermostatted spectrophotometer. Then add:

Reagent H (Myokinase)	0.10	-----
Reagent G (BSA)	-----	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.

CALCULATIONS:

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

3 = Total volume (in milliliters) of assay

df = Dilution factor

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

0.1 = Volume (in milliliter) of enzyme used

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

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

UNIT DEFINITION:

One unit will convert 2.0 μ moles of ADP to ATP + AMP per minute at pH 7.6 at 37°C.

FINAL ASSAY CONCENTRATION:

In a 3.00 ml reaction mix, the final concentrations are 58 mM glycylglycine, 2.0 mM adenosine 5'-diphosphate, 2.3 mM β -nicotinamide adenine dinucleotide phosphate, 10 mM magnesium chloride, 10 mM glucose, 2 units hexokinase, 1 unit glucose-6-phosphate dehydrogenase, 0.003% (w/v) bovine serum albumin, and 0.02 - 0.06 unit myokinase.

NOTES:

1. Unit Definition for Hexokinase: One unit will phosphorylate 1.0 μ mole of D-glucose per minute at pH 7.6 at 25°C.
2. Unit Definition for Glucose-6-Phosphate Dehydrogenase: 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.

REFERENCE:

Bergmeyer, H. U. (1974) *Methods of Enzymatic Analysis*,
2nd ed., Volume II, 486

NOTES:

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

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