Enzymatic Assay of CARBAMATE KINASE
(EC 2.7.2.2)

PRINCIPLE:

ADP + Carbamyl Phosphate $\xrightarrow{CK}$ ATP + NH_3 + CO_2

$_D$-Glucose + ATP $\xrightarrow[Mg^{++}]{Hexokinase}$ $_D$-Glucose 6-Phosphate + ADP

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

Abbreviations used:
ADP = Adenosine 5'-Phosphate
CK = Carbamate Kinase
ATP = Adenosine 5'-Triphosphate
ADP = Adenosine 5'-Diphosphate
ß-NADP = ß-Nicotinamide Adenine Dinucleotide Phosphate, Oxidized Form
G-6-PDH = Glucose 6-Phosphate Dehydrogenase
6-PG = 6-Phospho-$_D$-Gluconate
ß-NADPH = ß-Nicotinamide Adenine Dinucleotide Phosphate, Reduced Form

CONDITIONS: T = 37°C, pH = 8.3, A_{340nm}, Light path = 1 cm

METHOD: Continuous Spectrophotometric Rate Determination

REAGENTS:

A. 20 mM Tris HCl Buffer, pH 8.3 at 37°C
(Prepare 100 ml in deionized water using Trizma Base, Sigma Prod. No. T-1503. Adjust to pH 8.3 at 37°C with 1 M HCl.)

B. 10 mM ß-$_D$(+)Glucose, 34 mM Magnesium Chloride, 1.0 mM ß-Nicotinamide Adenine Dinucleotide Phosphate, Oxidized Form, and 5.0 mM Adenosine 5'-Diphosphate Solution, pH 8.3 at 37°C (Reagent Soln)
(Prepare 10 ml in Reagent A using ß-$_D$(+)Glucose, Sigma Prod. No. G-5250, Magnesium Chloride, Hexahydrate, Sigma Prod. No. M-0250, ß-Nicotinamide Adenine Dinucleotide Phosphate, Sigma Prod. No. N-7004, and Adenosine 5'-Diphosphate, Sodium Salt, Sigma Prod. No. A-6646. Adjust to pH 8.3 at 37°C with either 1 M HCl or 1 M NaOH if necessary.)

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REAGENTS:

C. 30 mM Carbamyl Phosphate Solution (Carbamyl Phosphate) (Immediately before use prepare 1 ml in deionized water using Carbamyl Phosphate, Dilithium Salt, Sigma Prod. No. C-5625. This solution is unstable. **PREPARE FRESH**.)

D. 0.05% (w/v) Bovine Serum Albumin Solution (BSA) (Prepare 10 ml in Reagent A using Albumin, Bovine, Sigma Prod. No. A-4503.)

E. Glucose-6-P-Dehydrogenase and Hexokinase Enzyme Solution (G-6-PDH/HK) (Immediately before use, prepare 1 ml of a solution containing 100 units/ml each of Glucose-6-Phosphate Dehydrogenase, Sigma Prod. No. G-5760, and Hexokinase, Sigma Prod. No. H-5500, in cold Reagent A.)

F. Carbamate Kinase Enzyme Solution (CK) (Immediately before use, prepare a solution containing 50 units/ml of Carbamate Kinase in cold deionized water. Then dilute to 0.3 - 0.4 unit/ml with Reagent D.)

PROCEDURE:

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

	Test	Blank
Reagent C (Carbamyl Phosphate)	0.10	0.10
Reagent B (Reagent Soln)	2.70	2.70
Reagent E (G-6-PDH/HK)	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 F (CK)	0.10	------
Reagent D (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.

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CALCULATIONS:

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

3 = Total volume (in milliliters) of assay
df = Dilution factor
6.22 = Millimolar extinction coefficient of ß-NADPH at 340 nm
0.1 = Volume (in milliliter) of carbamate kinase 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 form 1.0 µmole of ATP from ADP and carbamyl phosphate per minute at pH 8.3 at 37°C.

FINAL ASSAY CONCENTRATIONS:

In a 3.00 ml reaction mix, the final concentrations are 19 mM Tris, 9 mM ß-$_D$(+)glucose, 31 mM magnesium chloride, 0.9 mM ß-nicotinamide adenine dinucleotide phosphate, 4.5 mM adenosine 5'-diphosphate, 1 mM carbamyl phosphate, 0.002% bovine serum albumin, 10 units glucose-6-phosphate dehydrogenase, 10 units hexokinase, and 0.03 - 0.04 unit carbamate kinase.

REFERENCE:

Schimke, R.T., Berlin, C.M., Sweeney, E.W., and Carrol, W.R. (1966) *Journal of Biological Chemistry* **241**, 2228-2236

NOTES:

1. Hexokinase Unit Definition: One unit will phosphorylate 1.0 µmole of $_D$-glucose per minute at pH 7.6 at 25°C.

2. Glucose 6-Phosphate Dehydrogenase Unit Definition: One unit will oxidize 1.0 µmole of glucose 6-phosphate to 6-phospho-$_D$-gluconate per minute in the presence of NAD at pH 7.8 at 30°C.

NOTES: (continued)

3. This assay is based on the cited reference.

2. 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.