

**Enzymatic Assay of CREATINE PHOSPHOKINASE  
(EC 2.7.3.2)**

**PRINCIPLE:**

Phosphocreatine + ADP  $\xrightarrow{\text{Creatine Phosphokinase}}$  Creatine + ATP

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

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

Abbreviations used:

ADP = Adenosine 5'-Diphosphate

ATP = Adenosine 5'-Triphosphate

G-6-PDH = Glucose-6-Phosphate Dehydrogenase

$\beta$ -NADP =  $\beta$ -Nicotinamide Adenine Dinucleotide Phosphate,  
Oxidized Form

$\beta$ -NADPH =  $\beta$ -Nicotinamide Adenine Dinucleotide Phosphate,  
Reduced Form

6-PG = 6-Phospho-D-Gluconate

**CONDITIONS:** T = 30°C, pH = 7.4, A<sub>340nm</sub>, Light path = 1 cm

**METHOD:** Continuous Spectrophotometric Rate Determination

**REAGENTS:**

- A. 250 mM Glycylglycine Buffer with 0.10% (w/v) Bovine Serum Albumin, pH 7.4 at 30°C.  
(Prepare 100 ml in deionized water using Glycylglycine, Free Base, Sigma Prod. No. G-1002, and Albumin Bovine Serum, Sigma Prod. No. A-4503. Adjust to pH 7.4 at 30°C with 1 M NaOH.)
- B. 400 mM Phosphocreatine Solution  
(Prepare 10 ml in deionized water using Phosphocreatine, Disodium Salt Hydrate, Sigma Prod. No. P-6502.)
- C. 40 mM Adenosine 5'-Diphosphate Solution (ADP)  
(Prepare 10 ml in deionized water using Adenosine 5'-Diphosphate, Sodium Salt, Sigma Prod. No. A-8146.)

**Enzymatic Assay of CREATINE PHOSPHOKINASE  
(EC 2.7.3.2)**

**REAGENTS:** (continued)

- D. 1000 mM D-Glucose Solution  
(Prepare 10 ml in deionized water using  $\beta$ -D(+)Glucose, Sigma Prod. No. G-5250.)
- E. 20 mM  $\beta$ -Nicotinamide Adenine Dinucleotide Phosphate Solution ( $\beta$ -NADP)  
(Dissolve the contents of one 30 mg vial of  $\beta$ -Nicotinamide Adenine Dinucleotide Phosphate, Sodium Salt, Sigma Stock No. 240-330, in the appropriate volume of deionized water.)
- F. 300 mM Magnesium Acetate  
(Prepare 5 ml in deionized water using Magnesium Acetate, Tetrahydrate, Sigma Prod. No. M-0631.)
- G. Hexokinase Solution  
(Immediately before use, prepare a solution containing 300 units/ml of Hexokinase, Sigma Prod. No. H-4502, in cold deionized water.)
- H. Glucose-6-Phosphate Dehydrogenase Enzyme Solution (G-6-PDH)  
(Immediately before use, prepare a solution containing 10 units/ml of Glucose-6-Phosphate Dehydrogenase, Sigma Prod. No. G-6378, in cold deionized water.)
- I. Creatine Phosphokinase Enzyme Solution<sup>1</sup> (Creat PPK)  
(Immediately before use, prepare a solution containing 0.3 unit/ml of Creatine Phosphokinase in cold Reagent A.)

**PROCEDURE:**

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

|                               |       |
|-------------------------------|-------|
| Deionized Water               | 36.00 |
| Reagent A (Buffer)            | 10.00 |
| Reagent B (Phosphocreatine)   | 2.00  |
| Reagent C (ADP)               | 2.00  |
| Reagent D (D-Glucose)         | 2.00  |
| Reagent E ( $\beta$ -NADP)    | 1.20  |
| Reagent F (Magnesium Acetate) | 0.80  |

Mix and adjust to pH 7.4 at 30°C with 1 M NaOH.

**Enzymatic Assay of CREATINE PHOSPHOKINASE  
(EC 2.7.3.2)**

**PROCEDURE:** (continued)

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

|                        | <u>Test</u> | <u>Blank</u> |
|------------------------|-------------|--------------|
| Reaction Cocktail      | 2.70        | 2.70         |
| Reagent G (Hexokinase) | 0.10        | 0.10         |
| Reagent H (G-6-PDH)    | 0.10        | 0.10         |

Mix by inversion and equilibrate to 30°C. Monitor the  $A_{340\text{nm}}$  until constant, using a suitably thermostatted spectrophotometer. Then add:

|                       |       |      |
|-----------------------|-------|------|
| Reagent A (Buffer)    | ----- | 0.10 |
| Reagent I (Creat PPK) | 0.10  | ---  |
|                       |       | ---  |

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

**CALCULATIONS:**

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

3.0 = Total volume (in milliliters) of assay

df = Dilution factor

6.22 = Millimolar extinction coefficient of  $\beta$ -NADPH at 340nm

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 transfer 1.0  $\mu\text{mole}$  of phosphate from phosphocreatine to ADP per minute at pH 7.4 at 30°C.

**Enzymatic Assay of CREATINE PHOSPHOKINASE  
(EC 2.7.3.2)**

**FINAL ASSAY CONCENTRATION:**

In a 3.00 ml reaction mix, the final concentrations are 50 mM glycylglycine, 0.020% (w/v) bovine serum albumin, 13 mM phosphocreatine, 1.3 mM adenosine 5'-diphosphate, 33 mM D-glucose, 0.40 mM  $\beta$ -nicotine adenine dinucleotide phosphate, oxidized form, 4.0 mM magnesium acetate, 30 units hexokinase, 1.0 unit glucose-6-phosphate dehydrogenase and 0.03 unit creatine phosphokinase.

**REFERENCES:**

Noda, L., Nihei, T. and Morales, M.F. (1960) *J. Biol. Chem.* **235**, 2830-2834

Forster, G., Bernt, E. and Bergmeyer, H.U. (1974) in *Methods of Enzymatic Analysis* (Bergmeyer, H.U. ed) Volume II, 2nd ed., 789-793, Academic Press, Inc., New York, NY

**NOTES:**

1. Ammonium sulfate and chloride are strong inhibitors of Creatine Phosphokinase.
2. This assay is based on the cited reference.
3. Hexokinase Unit Definition: One unit will phosphorylate 1.0  $\mu$ mole of D-glucose per minute at pH 7.6 at 25°C.
4. Glucose-6-Phosphate Dehydrogenase Unit Definition: One unit will oxidize 1.0  $\mu$ 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.
5. 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.**