

**Determination of the Concentration and
Molecular Weight of PHOSPHOCREATINE**

PRINCIPLE:

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

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

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

Abbreviations used:

ADP = Adenosine 5'-Diphosphate

ATP = Adenosine 5'-Triphosphate

β -NADP = β -Nicotinamide Adenine Dinucleotide Phosphate,
Oxidized Form

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

6-PG = 6-Phospho-D-Gluconate

β -NADPH = β -Nicotinamide Adenine Dinucleotide Phosphate,
Reduced Form

CONDITIONS: T = 30°C, pH = 7.4, A_{340nm}, Light path = 1 cm

METHOD: Spectrophotometric Determination

REAGENTS:

- A. 250 mM Glycylglycine Buffer with 0.1% (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, Sigma Prod. No. A-4503. Adjust to pH 7.4 at 30°C with 1 M NaOH.)
- B. 20 mM Adenosine 5'-Diphosphate Solution (ADP)
(Prepare 1 ml in deionized water using Adenosine 5'-Diphosphate, Sodium Salt, Sigma Prod. No. A-6521.)
- C. 1 M D-Glucose Solution (Glucose)
(Prepare 1 ml in deionized water using β -D(+)Glucose, Sigma Prod. No. G-5250.)

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REAGENTS: (continued)

- D. 20 mM β -Nicotinamide Adenine Dinucleotide Phosphate Solution (β -NADP)
(Dissolve the contents of one 30 mg vial of β -Nicotinamide Adenine Dinucleotide Phosphate, Sodium Salt, Sigma Stock No. 240-330, in the appropriate volume of deionized water. **PREPARE FRESH.**)
- E. 300 mM Magnesium Chloride Solution ($MgCl_2$)
(Prepare 1 ml in deionized water using Magnesium Chloride, Hexahydrate, Sigma Prod No. M-0250.)
- F. Glucose-6-Phosphate Dehydrogenase and Hexokinase Enzyme Solution (G-6-PDH/HK)
(Immediately before use, prepare a solution containing 50 units/ml of Hexokinase and Glucose-6-Phosphate Dehydrogenase, Sigma Prod. No. H-8629, in cold Reagent A. The 50 units are based on the Glucose-6-Phosphate Dehydrogenase Activity¹.)
- G. Creatine Phosphokinase Enzyme Solution (CPK)
(Immediately before use, prepare a solution containing 50 units/ml of Creatine Phosphokinase, Sigma Prod. No. C-3755, in cold Reagent A.)
- H. Phosphocreatine Solution (Phosphocreatine)
Immediately before using, weigh approximately 2 mg of Phosphocreatine and dissolve in 25 ml of deionized water.)

PROCEDURE:

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

	<u>Test</u>	<u>Blank</u>
Reagent A (Buffer)	0.60	0.60
Reagent B (ADP)	0.10	0.10
Reagent C (Glucose)	0.10	0.10
Reagent D (β -NADP)	0.06	0.06
Reagent E ($MgCl_2$)	0.04	0.04
Deionized Water	1.00	2.00
Reagent F (G-6-PDH/HK)	0.10	0.10
Reagent H (Phosphocreatine)	1.00	-----

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PROCEDURE:

Mix by inversion and equilibrate to 30°C using a suitably thermostatted spectrophotometer. Record the initial $A_{340\text{nm}}$ for the Test and Blank. Then add:

	<u>Test</u>	<u>Blank</u>
Reagent G (CPK)	0.10	0.10

Immediately mix by inversion and allow the reaction to proceed to completion (approximately 5 minutes). Record the final $A_{340\text{nm}}$ for both the Test and Blank.

CALCULATION:

$$r A = A_f - A_i$$

A_f = Final Absorbance

A_i = Initial Absorbance

$$\text{Micromoles Phosphocreatine/weighed sample} = \frac{(r A_{\text{Test}} - r A_{\text{Blank}})(3.1)(25)}{(6.22)}$$

3.1 = Total volume (in milliliters) of assay

25 = Dilution factor

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

$$\text{Apparent Molecular Weight} = \frac{\text{mg sample weighed} \times 1000}{\mu\text{moles PC/ weighed sample}}$$

1000 = Conversion factor from mg to μg

PC = Phosphocreatine

FINAL ASSAY CONCENTRATION:

In a 3.10 ml reaction mix, the final concentrations are 65 mM glycylglycine, 0.03% (w/v) bovine serum albumin, 0.65 mM adenosine 5'-diphosphate, 32 mM D-glucose, 0.4 mM β -nicotinamide adenine dinucleotide phosphate, 4 mM magnesium chloride, 5 units creatine phosphokinase, 10 units hexokinase, 5 units glucose 6-phosphate dehydrogenase, and varying amounts of phosphocreatine.

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REFERENCE:

Szasz, G., Gruber, W. and Bernt, E. (1976) *Clinical Chemistry* **22**, 650-656

NOTES:

1. Hexokinase and Glucose 6-Phosphate Dehydrogenase, Sigma Prod. No. H-8629, has twice as many units of Hexokinase as it does Glucose 6-Phosphate Dehydrogenase.
2. Hexokinase Unit Definition: One unit will phosphorylate 1.0 μ mole of D-glucose per minute at pH 7.6 at 25°C.
3. 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 7.4 at 25°C.
4. Creatine Phosphokinase Unit Definition: One unit will transfer 1.0 μ mole of phosphate from phosphocreatine to ADP per minute at pH 7.4 at 30°C.
5. This assay is based on the cited reference.
6. 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.