

**Enzymatic Assay of CREATININASE  
(EC 3.5.2.10)**

**PRINCIPLE:**

Creatinine + H<sub>2</sub>O  $\xrightarrow{\text{Creatininase}}$  Creatine

**CONDITIONS:** T = 37°C, pH = 6.5, A<sub>525nm</sub>, Light path = 1 cm

**METHOD:** Colorimetric

**REAGENTS:**

- A. 300 mM Potassium Phosphate Buffer, pH 6.5 at 37°C  
(Prepare 100 ml in deionized water using Potassium Phosphate, Monobasic, Anhydrous, Sigma Prod. No. P-5379. Adjust to pH 6.5 at 37°C with Reagent B.)
- B. 300 mM Potassium Phosphate Solution  
(Prepare 100 ml in deionized water using Potassium Phosphate, Dibasic, Trihydrate, Sigma Prod. No. P-5504.)
- C. 10 mM Potassium Phosphate Buffer, pH 8.0 at 37°C  
(Enzyme Diluent)  
(Prepare 25 ml in deionized water using Reagent A. Adjust to pH 8.0 at 37°C with Reagent B.)
- D. 0.1 mM Mercury Chloride and 189 mM Sodium Carbonate Solution (Stop Rgt)  
(Prepare 100 ml in deionized water using Mercuric Chloride, Sigma Prod. No. M-1136 and Sodium Carbonate, Anhydrous, Sigma Prod. No. S-2127.)
- E. 139 mM a-Naphthol Solution (a-Naphthol)  
(Prepare 100 ml in Reagent F using a-Naphthol, Sigma Prod. No. N-1000.)
- F. 95% (v/v) Ethanol (EtOH)  
(Prepare 100 ml in deionized water using 200 Proof USP Ethyl Alcohol, available from Quantum Chemical Company.)

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**REAGENTS:** (continued)

- G. 300 mM Sodium Hydroxide and 300 mM Sodium Carbonate Solution (Alk Soln)  
(Prepare 100 ml in deionized water using Sodium Hydroxide, Anhydrous, Sigma Prod. No. S-5881 and Sodium Carbonate, Anhydrous, Sigma Prod. No. S-2127.)
- H. 0.05% (v/v) Diacetyl Solution (Diacet)  
(Prepare 50 ml in deionized water using Diacetyl, Sigma Prod. No. D-3634.)
- I. 100 mM Creatinine Substrate Solution (Creatinine)  
(Prepare 25 ml in deionized water using Creatinine, Free Base, Anhydrous, Sigma Prod. No. C-4255.)
- J. 4 mM Creatine Standard Solution (Creat Std)  
(Prepare 10 ml in Reagent C using Creatine, Hydrate, Sigma Prod. No. C-3630.)
- K. Creatininase Enzyme Solution  
(Immediately before use, prepare a solution containing 5 - 10 units/ml of Creatininase in cold Reagent C.)

**PROCEDURE:**

Step 1:

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

	<u>Test</u>	<u>Blank</u>
Reagent A (Buffer)	0.10	0.10
Reagent I (Creatinine)		0.80
		0.80

Mix by swirling and equilibrate to 37°C. Then add:

Reagent K (Enzyme Soln)		0.10
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Immediately mix by swirling and incubate at 37°C for exactly 10 minutes. Then add:

Reagent D (Stop Rgt)	2.00	2.00
Reagent K (Enzyme Soln)		-----
		0.10

Mix by swirling.

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**PROCEDURE:**

Step 2:

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

	<u>Test</u>	<u>Blank</u>
Deionized Water	0.90	0.90
Reagent E (a-Naphthol)	0.50	0.50
Reagent G (Alk Soln)	0.50	0.50
Reagent H (Diacet)	0.50	0.50

Mix by swirling and then add:

Test Solution	0.10	-----
Blank Solution	-----	0.10

Mix by swirling and incubate at 25°C for 1 hour. Then add:

Deionized Water	2.50	2.50
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Mix by swirling.

Transfer the Test, Blank, Standards and Standard Blank to suitable cuvettes and read the absorbance at 525nm.

**COLOR DEVELOPMENT:**

Standard Curve:

Prepare a standard curve by pipetting (in milliliters) the following reagents into suitable containers:

	<u>Std 1</u>	<u>Std 2</u>	<u>Std 3</u>	<u>Std 4</u>	<u>Std 5</u>	<u>Std Blank</u>
Deionized Water	0.98	0.96	0.94	0.92	0.90	1.00
Reagent E (a-Naphthol)	0.50	0.50	0.50	0.50	0.50	0.50
Reagent G (Alk Soln)	0.50	0.50	0.50	0.50	0.50	0.50
Reagent H (Diacet)	0.50	0.50	0.50	0.50	0.50	0.50

Mix by swirling and then add:

Reagent J (Creat Std)	0.02	0.04	0.06	0.08	0.10	-----
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Mix by swirling and incubate at 25°C for one hour. Then add:

Deionized Water	2.50	2.50	2.50	2.50	2.50	2.50
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Mix by swirling. Transfer the Standards and Standard Blank to suitable cuvettes and read the absorbance at 525nm.

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**CALCULATIONS:**

Standard Curve:

$$r A_{525\text{nm}} \text{ Standard} = A_{525\text{nm}} \text{ Standard} - A_{525\text{nm}} \text{ Standard Blank}$$

Plot the  $r A_{525\text{nm}}$  Standard vs  $\mu\text{moles creatine}$ .

Sample Determination:

$$r A_{525\text{nm}} \text{ Sample} = A_{525\text{nm}} \text{ Test} - A_{525\text{nm}} \text{ Sample Blank}$$

Determine the  $\mu\text{moles}$  of creatinine hydrolyzed using the Standard Curve.

$$\text{Units/ml enzyme} = \frac{(\mu\text{moles Creatinine hydrolyzed})(3)(\text{df})}{(10)(0.1)(0.1)}$$

3 = Volume (in milliliters) of stopped reaction

df = Dilution factor

10 = Time of assay (in minutes) as per the Unit Definition

0.1 = Volume (in milliliters) of stopped reaction used in  
Colorimetric Determination

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 hydrolyze 1.0  $\mu\text{mole}$  of creatinine to creatine per minute at pH 6.5 at 37°C.

**FINAL ASSAY CONCENTRATION:**

In a 1.00 ml reaction mix, the final concentrations are 31 mM potassium phosphate, 80 mM creatinine, and 0.5 - 1.0 unit creatininase.

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**NOTES:**

1. 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.**