

**Enzymatic Assay of CREATINASE
(EC 3.5.3.3)**

PRINCIPLE:

Creatine + H₂O $\xrightarrow{\text{Creatinase}}$ Sarcosine + Urea

Urea + DAB \longrightarrow Yellow Dye Product

Abbreviation used:

DAB = p-Dimethylaminobenzaldehyde

CONDITIONS: T = 37°C, pH = 7.5, A_{435nm}, Light path = 1 cm

METHOD: Spectrophotometric Stopped Rate Determination

REAGENTS:

- A. 50 mM Potassium Phosphate Buffer, pH 7.5 at 37°C
(Prepare 100 ml in deionized water using Potassium Phosphate, Monobasic, Anhydrous, Sigma Prod. No P-5379. Adjust to pH 7.5 at 37°C with 5 M NaOH.)
- B. 100 mM Creatine Solution (Creatine)
(Prepare 10 ml in Reagent A using Creatine, Hydrate, Sigma Prod. No. C-3630. Adjust to pH 7.5 at 37°C, if necessary, with either 1 M HCl or 1 M NaOH.)
- C. Dimethyl Sulfoxide
(Use Dimethyl Sulfoxide, Sigma Prod. No. D-8779.)
- D. Hydrochloric Acid
(Use Hydrochloric Acid, Sigma Prod. No. H-7020.)
- E. 117 mM p-Dimethylaminobenzaldehyde Solution (DMAB)
(Prepare by dissolving 1 g of p-Dimethylaminobenzaldehyde, Sigma Prod. No. D-2004, in 50 ml of Reagent C. Then carefully add 7.5 ml of Reagent D and mix by swirling. Protect from light. **PREPARE FRESH.**)
- F. Creatinase Enzyme Solution
(Immediately before use, prepare a solution containing 2 - 4 units/ml of Creatinase in cold Reagent A.)

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PROCEDURE:

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

	<u>Test</u>	<u>Blank</u>
Reagent B (Creatine)	0.90	0.90

Equilibrate to 37°C using a suitably thermostatted spectrophotometer. Then add:

Reagent F (Enzyme Solution)	0.10	-----
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Immediately mix by inversion and incubate at 37°C for exactly 10 minutes. Then add:

Reagent E (DMAB)	2.00	2.00
Reagent F (Enzyme Solution)	-----	0.10

Mix by swirling and let stand for 20 minutes at 25°C. Record the $A_{435\text{nm}}$ for both the Test and Blank.

CALCULATIONS:

$$\text{Units/ml enzyme} = \frac{(A_{435\text{nm}} \text{ Test} - A_{435\text{nm}} \text{ Blank})(3)(\text{df})}{(0.321)(10)(0.1)}$$

3 = Total volume (in milliliters) of assay

df = Dilution factor

0.321 = Millimolar extinction coefficient¹ of the yellow dye

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

0.1 = Volume (in milliliter) 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 μmole of creatine to urea and

sarcosine per minute at pH 7.5 at 37°C.

FINAL ASSAY CONCENTRATIONS:

In a 1.00 ml reaction mix, the final concentrations are 50 mM potassium phosphate, 90 mM creatine, and 0.2 - 0.4 unit creatinase.

REFERENCE:

Dawson, R.M.C., Elliott, D.C., Elliott, W.H., Jones, K.M. (1987) *Data for Biochemical Research*, pp. 460, Oxford University Press, Oxford

NOTES:

1. The millimolar extinction coefficient of the yellow dye product at 435 nm was determined by the supplier of the enzyme to Sigma.
2. This assay is based on the cited reference.
3. 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.