Enzymatic Assay of CREATINASE
(EC 3.5.3.3)

PRINCIPLE:
Creatine + H$_2$O $\xrightarrow{\text{Creatinase}}$ Sarcosine + Urea
Urea + DAB $\xrightarrow{}$ Yellow Dye Product

Abbreviation used:
DAB = p-Dimethylaminobenzaldehyde

CONDITIONS:  T = 37°C, pH = 7.5, A$_{435\text{nm}}$, 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. 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. 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.)

D.  Hydrochloric Acid
(Use Hydrochloric Acid.)

E.  117 mM p-Dimethylaminobenzaldehyde Solution (DMAB)
(Prepare by dissolving 1 g of p-Dimethylaminobenzaldehyde, 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:

<table>
<thead>
<tr>
<th></th>
<th>Test</th>
<th>Blank</th>
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<tbody>
<tr>
<td>Reagent B (Creatine)</td>
<td>0.90</td>
<td>0.90</td>
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Equilibrate to 37°C using a suitably thermostatted spectrophotometer. Then add:

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

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<tr>
<td>Reagent E (DMAB)</td>
<td>2.00</td>
<td>2.00</td>
</tr>
<tr>
<td>Reagent F (Enzyme Solution)</td>
<td>------</td>
<td>0.10</td>
</tr>
</tbody>
</table>

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:

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

3 = Total volume (in milliliters) of assay
\(\text{df}\) = Dilution factor
0.321 = Millimolar extinction coefficient\(^1\) of the yellow dye
10 = Time (in minutes) of assay as per the Unit Definition
0.1 = Volume (in milliliter) of enzyme used

Units/mg solid = \[
\frac{\text{mg solid/ml enzyme}}{\text{units/ml enzyme}}
\]

Units/mg protein = \[
\frac{\text{mg protein/ml enzyme}}{\text{units/ml enzyme}}
\]

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:**


**NOTES:**

1. The millimolar extinction coefficient of the yellow dye product at 435 nm was determined by the supplier of the enzyme.

2. This assay is based on the cited reference.

This procedure is for informational purposes.