Enzymatic Assay of CREATININE DEIMINASE
(EC 3.5.4.21)

PRINCIPLE:
Creatinine + H₂O \(\text{Creatinine Deiminase}\) > N-Methylhydantoin + NH₃
NH₄⁺ + a-Ketoglutarate + ß-NADPH \(\text{GDH}\) > Glutamate + H₂O + ß-NADP

Abbreviations used:
ß-NADPH = ß-Nicotinamide Adenine Dinucleotide Phosphate, Reduced Form
GDH = L-Glutamic Dehydrogenase (NADP)
ß-NADP = ß-Nicotinamide Adenine Dinucleotide Phosphate, Oxidized Form

CONDITIONS: T = 37°C, pH = 7.5, A₃⁴⁰nm, Light path = 1 cm

METHOD: Continuous Spectrophotometric 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 1 M KOH.)

B. 50 mM Creatinine Solution (Creatinine)
   (Prepare 25 ml in Reagent A using Creatinine, Free Base, Anhydrous.)

C. 3.0 mM ß-Nicotinamide Adenine Dinucleotide Phosphate, Reduced Form Solution (ß-NADPH)
   (Dissolve the contents of one 5 mg vial of ß-Nicotinamide Adenine Dinucleotide Phosphate, Reduced Form, Tetrasodium Salt, in the appropriate volume of Reagent A. PREPARE FRESH.)

D. 10.0 mM a-Ketoglutarate Solution (a-KG)
   (Prepare 3 ml in Reagent A using a-Ketoglutaric Acid, Free Acid, PREPARE FRESH.)

E. L-Glutamic Dehydrogenase (NADP) Enzyme Solution (GDH)
   (Immediately before use, prepare a solution containing 1000 units/ml of L-Glutamic Dehydrogenase (NADP), in cold deionized water.)
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REAGENTS:

F. Creatinine Deiminase Enzyme Solution (Creat Deiminase)
(Immediately before use, prepare a solution containing
0.06 - 0.12 unit/ml of Creatinine Deiminase in cold
Reagent A.)

PROCEDURE:

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

<table>
<thead>
<tr>
<th></th>
<th>Test</th>
<th>Blank</th>
</tr>
</thead>
<tbody>
<tr>
<td>Reagent B (Creatinine)</td>
<td>2.40</td>
<td>2.40</td>
</tr>
<tr>
<td>Reagent C (β-NADPH)</td>
<td>0.30</td>
<td>0.30</td>
</tr>
<tr>
<td>Reagent D (a-KG)</td>
<td>0.30</td>
<td>0.30</td>
</tr>
<tr>
<td>Reagent E (GDH)</td>
<td>0.05</td>
<td>0.05</td>
</tr>
</tbody>
</table>

Mix by inversion and equilibrate to 37°C. Monitor the
A\textsubscript{340nm} until constant, using a suitably thermostatted
spectrophotometer. Then add:

<table>
<thead>
<tr>
<th></th>
<th>Test</th>
<th>Blank</th>
</tr>
</thead>
<tbody>
<tr>
<td>Reagent F (Creat Deiminase)</td>
<td>0.10</td>
<td>------</td>
</tr>
<tr>
<td>Reagent A (Buffer)</td>
<td>------</td>
<td>0.10</td>
</tr>
</tbody>
</table>

Immediately mix by inversion and record the decrease in
A\textsubscript{340nm} for approximately 5 minutes. Obtain the \( \frac{r_{A_{340nm}}}{\text{minute}} \) using the maximum linear rate for both the Test and Blank.

CALCULATIONS:

\[
\text{Units/ml enzyme} = \frac{(r_{A_{340nm}/\text{min Test}} - r_{A_{340nm}/\text{min Blank}})(3.15)(df)}{(6.22)(0.1)}
\]

3.15 = Total volume (in milliliters) of assay
df = Dilution factor
6.22 = Millimolar extinction coefficient of β-NADPH
at 340 nm
0.1 = Volume (in milliliter) of enzyme used

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

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

One unit will hydrolyze 1.0 µmole of creatinine to N-methylhydantoin and NH₃ per minute at pH 7.5 at 37°C in a coupled system with l-glutamic dehydrogenase.

FINAL ASSAY CONCENTRATIONS:

In a 3.15 ml reaction mix, the final concentrations are 49 mM potassium phosphate, 38 mM creatinine, 0.29 mM β-nicotinamide adenine dinucleotide phosphate, reduced form, 0.95 mM α-ketoglutaric acid, 50 units l-glutamic dehydrogenase (NADP), and 0.006 – 0.012 unit creatinine deiminase.

REFERENCE:


NOTES:

1. This assay is based on the cited reference.

2. l-Glutamic Acid Dehydrogenase (NADP) Unit Definition: One unit will reduce 1.0 µmole of α-ketoglutarate to l-glutamate per minute at pH 8.3 at 30°C in the presence of ammonium ions and NADPH.

3. Where Product or Stock numbers are specified, equivalent reagents may be substituted.

This procedure is for informational purposes. For a current copy of our quality control procedure contact our Technical Service Department.