Enzymatic Assay of ACONITASE
(EC 4.2.1.3)

PRINCIPLE:

Aconitase
Citrate $\xrightarrow{\text{Fe}^{2+}, \text{Cysteine}}$ Isocitrate

Isocitrate + β-NADP $\xrightarrow{\text{ICD}}$ a-Ketoglutarate + CO$_2$ + β-NADPH

Abbreviations:
- β-NADP = β-Nicotinamide Adenine Dinucleotide Phosphate, Oxidized Form
- β-NADPH = β-Nicotinamide Adenine Dinucleotide Phosphate, Reduced Form
- ICD = Isocitric Dehydrogenase

CONDITIONS: $T = 25^\circ C$, pH = 7.4, $A_{340nm}$, Light path = 1 cm

METHOD: Continuous Spectrophotometric Rate Determination

REAGENTS:

A. 100 mM Tris Buffer, pH 7.4 at 25°C
(Prepare 100 ml in deionized water using Trizma Base. Adjust to pH 7.4 at 25°C with 1 M HCl.)

B. 2 mM Citric Acid Solution (Cit)
(Prepare 20 ml in deionized water using Citric Acid Free Acid, Monohydrate. Adjust to pH 7.4 with 100 mM NaOH.)

C. 5.4 mM β-Nicotinamide Adenine Dinucleotide Phosphate Solution (β-NADP)
(Dissolve the contents of a 5 mg vial of β-Nicotinamide Adenine Dinucleotide Phosphate, Sodium Salt, in the appropriate volume of deionized water or prepare 1 ml in deionized water using β-Nicotinamide Adenine Dinucleotide Phosphate, Sodium Salt. PREPARE FRESH.)
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REAGENTS: (continued)

D. 1 mM Ferrous Ammonium Sulfate Solution (Fe(NH₄)₂(SO₄)₂) 
(Prepare 1 ml in deionized water using Ferrous Ammonium Sulfate, Hexahydrate. 
PREPARE FRESH.)

E. 20 mM Manganese Sulfate (MnSO₄) 
(Prepare 2 ml in deionized water using Manganese Sulfate.)

F. 50 mM L-Cysteine Solution, pH 7.4 at 25°C (Cys) 
(Prepare 5 ml in deionized solution using L-Cysteine Hydrochloride. Adjust to pH 7.4 at 25°C with 
1 M NaOH. PREPARE FRESH.)

G. Activation Buffer 
(Prepare by combining 4 ml of Reagent A, 0.10 ml of Reagent D, and 0.2 ml of Reagent F. 
Store at 0°C.)

H. Isocitric Dehydrogenase Enzyme Solution (IsoDH) 
(Immediately before use, prepare a solution containing 14 units/ml in deionized water using 
Isocitric Dehydrogenase.)

I. Aconitase Enzyme 
(Use 15 mg.)

PROCEDURE:

Prepare activated Aconitase enzyme solution by combining the following reagents in a suitable vial:

Reagent I (Enzyme) 15 mg
Reagent G (Activation Buffer) 2.15 ml

Mix and incubate at 0°C for 1 hour.

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>Deionized Water</td>
<td>1.45</td>
<td>1.45</td>
</tr>
<tr>
<td>Reagent A (Buffer)</td>
<td>1.00</td>
<td>1.00</td>
</tr>
<tr>
<td>Reagent B (Cit)</td>
<td>0.10</td>
<td>0.10</td>
</tr>
<tr>
<td>Reagent C (β-NADP)</td>
<td>0.10</td>
<td>0.10</td>
</tr>
<tr>
<td>Reagent E (MnSO₄)</td>
<td>0.20</td>
<td>0.20</td>
</tr>
<tr>
<td>Reagent H (IsoDH)</td>
<td>0.05</td>
<td>0.05</td>
</tr>
</tbody>
</table>
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PROCEDURE: (continued)

Mix by inversion and equilibrate at 25°C. Monitor the $A_{340\text{nm}}$ until constant, using a suitably thermostatted spectrophotometer.

Then add:

<table>
<thead>
<tr>
<th>Test</th>
<th>Blank</th>
</tr>
</thead>
<tbody>
<tr>
<td>Activated Aconitase Enzyme Solution</td>
<td>0.10</td>
</tr>
<tr>
<td>Reagent G (Activation Buffer)</td>
<td>0.10</td>
</tr>
</tbody>
</table>

Immediately mix by inversion and record the increase in $A_{340\text{nm}}$ for approximately 5 minutes. Obtain $r_{A_{340\text{nm}}}$/minute using the maximum linear rate for both the Test and Blank.

CALCULATIONS:

\[
\text{Units/ml enzyme} = \frac{(r_{A_{340\text{nm}}}/\text{min Test} - r_{A_{340\text{nm}}}/\text{min Blank})(3)(\text{df})}{6.22(0.1)}
\]

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

\[
\text{Units/g solid} = \frac{\text{units/ml enzyme (1000)}}{\text{mg/ml enzyme}}
\]

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

UNIT DEFINITION:

One unit will convert 1.0 µmole of citrate (via cisÅaconitate) to isocitrate per minute at pH 7.4 at 25°C.

FINAL ASSAY CONCENTRATIONS:

In a 3.00 ml reaction mix, the final concentrations are 36 mM Tris, 0.07 mM citric acid, 0.18 mM β-nicotinamide adenine dinucleotide phosphate, 1.3 mM manganese sulfate, 0.0008 mM ferrous ammonium sulfate, 0.08 mM L-cysteine, 0.7 unit isocitric dehydrogenase, and 0.70 mg aconitase.
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REFERENCE:


NOTES:

1. This assay is based on the cited reference.

2. Where our 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.