Enzymatic Assay of α-ACETOLACTATE DECARBOXYLASE (EC 4.1.1.5)

**PRINCIPLE:**

\[
\text{2-Acetolactate} \xrightarrow{\alpha\text{-Acetolactate Decarboxylase}} \text{Acetoin + CO}_2
\]

**CONDITIONS:** \( T = 30^\circ C, \ \text{pH} = 6.0, \ A_{522\text{nm}} \), Light path = 1 cm

**METHOD:** Stopped Spectrophotometric Rate Determination

**REAGENTS:**

A. 50 mM MES Buffer, pH 6.0 at 30°C  
(Prepare 100 ml in deionized water using MES Free Acid, Monohydrate, Sigma Stock No. M-5287. Adjust to pH 6.0 at 30°C with 1 M NaOH.)

B. 15% (w/v) Brij 35 Solution (Brij 35)  
(Prepare 2 ml in deionized water using Brij 35 Solution, 30% w/v solution, Sigma Stock No. 430AG-6. Heat to no more than 60°C to dissolve.)

C. 50 mM MES Buffer, pH 6.0 at 30°C with 0.005% (w/v) Brij 35 and 600 mM Sodium Chloride (Buffer C)  
(Prepare 500 ml by dissolving 4.9 g of MES, Free Acid, Monohydrate, Sigma Prod. No. M-5287 and 17.5 g of Sodium Chloride, Sigma Prod. No. S-9625 in approximately 450 ml of deionized water. Add 1.7 ml of Reagent B and adjust to pH 6.0 at 30°C with 1 M NaOH.)

D. 10 mM α-Acetolactate Substrate Solution (α-AL)  
(Prepare by diluting 0.05 ml of Ethyl 2-acetoxy-2-methylacetoacetate, Aldrich Stock No. 22,039-6 with 1.5 ml of deionized water and 1.5 ml of 1 N NaOH. Stir 20 minutes, Q.S. to 20 ml with Reagent A. Adjust to pH 6.0 with 0.5 M HCl and then Q.S. to 25 ml with Reagent A.)

E. Color Reagent Solution (Color Rgt)  
(Prepare 250 ml by dissolving 2.5 g of a-Naphthol, Sigma Prod. No. N-1000 and 0.25 g of Creatine, Hydrate, Sigma Prod. No. C-3630 in 1 N NaOH (use a minimum amount). Q.S. to 250 ml with 1 N NaOH.)
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REAGENTS: (continued)

F. 2.27 mM Acetoin Stock Solution
(Prepare 100 ml in deionized water using 3-Hydroxy-2-Butanone, Aldrich Stock
No. A1,795-1.)

H. α-Acetolactate Decarboxylase Enzyme Solution
(Immediately before use, prepare a solution containing approximately 0.03 unit/ml of α-
Acetolactate Decarboxylase in Reagent C.)

PROCEDURE:

Part I

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

<table>
<thead>
<tr>
<th>Enzyme</th>
<th>Test</th>
<th>Blank</th>
</tr>
</thead>
<tbody>
<tr>
<td>Reagent D (a-AL)</td>
<td>0.20</td>
<td>0.20</td>
</tr>
<tr>
<td>Reagent H (Enz Sol)</td>
<td>0.20</td>
<td>-----</td>
</tr>
<tr>
<td>Reagent C (Buffer C)</td>
<td>-----</td>
<td>-----</td>
</tr>
</tbody>
</table>

Incubate for 20 minutes at 30°C in a water bath. Then add:

<table>
<thead>
<tr>
<th>Enzyme</th>
<th>Test</th>
<th>Blank</th>
</tr>
</thead>
<tbody>
<tr>
<td>Reagent E (Color Rgt)</td>
<td>4.50</td>
<td>4.50</td>
</tr>
<tr>
<td>Reagent H (Enz Sol)</td>
<td>-----</td>
<td>0.20</td>
</tr>
</tbody>
</table>

Incubate for 40 minutes at room temperature. Transfer the solutions to suitable cuvettes and record
the $A_{522\text{nm}}$ for the Tests and Blanks using a suitable spectrophotometer.

Part II Standard Curve

Pipette (in milliliters) the following reagents into suitable 10 ml plastic tubes:

<table>
<thead>
<tr>
<th>Std 1</th>
<th>Std 2</th>
<th>Std 3</th>
<th>Std 4</th>
<th>Std 5</th>
<th>Blank</th>
</tr>
</thead>
<tbody>
<tr>
<td>Acetoin</td>
<td>0.020</td>
<td>0.040</td>
<td>0.080</td>
<td>0.120</td>
<td>0.160</td>
</tr>
<tr>
<td>Milli-Q</td>
<td>0.380</td>
<td>0.360</td>
<td>0.320</td>
<td>0.280</td>
<td>0.240</td>
</tr>
<tr>
<td>Color Solution</td>
<td>4.50</td>
<td>4.50</td>
<td>4.50</td>
<td>4.50</td>
<td>4.50</td>
</tr>
</tbody>
</table>

Color develops in 40 minutes at room temperature. Transfer the solutions to suitable cuvettes and record
the $A_{522\text{nm}}$ for the Standards and Standard Blank using a suitable spectrophotometer.
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CALCULATIONS:

Standard Curve:

\[ r \, A_{\text{522nm, Standard}} = A_{\text{522nm, Standard}} - A_{\text{522nm, Standard Blank}} \]

Prepare a standard curve by plotting the \( r A_{\text{522nm}} \) of the Standard versus \( \mu \)moles of acetoin.

Sample Determination:

\[ r \, A_{\text{522nm, Test}} = A_{\text{522nm, Test}} - A_{\text{522nm, Test Blank}} \]

Determine the micromoles of acetoin produced using the standard curve.

\[
\text{Units/ml enzyme} = \frac{(\mu \text{moles of acetoin produced})(4.9)(\text{df})}{(20)(0.2)}
\]

4.9 = Total volume (in milliliters) of assay
\( \text{df} \) = Dilution factor
20 = Time (in minutes) of assay
0.2 = 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 form one \( \mu \)mole of acetoin from acetolactate per minute at pH 6.0 at 30°C.

FINAL ASSAY CONCENTRATION:

In a 0.40 ml reaction mix, the final concentrations are 5 mM DL-acetolactate, 48 mM MES (approximately) and 0.006 unit a-acetolactate decarboxylase.

REFERENCE:

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NOTES:

1. This assay is based on the cited reference.

2. 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.