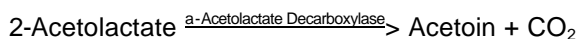


**Enzymatic Assay of α -ACETOLACTATE DECARBOXYLASE
(EC 4.1.1.5)**

PRINCIPLE:



CONDITIONS: T = 30°C, 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 α -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:

	<u>Test</u>	<u>Enzyme Blank</u>
Reagent D (α -AL)	0.20	0.20
Reagent H (Enz Sol)	0.20	-----
Reagent C (Buffer C)	-----	-----

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

Reagent E (Color Rgt)	4.50	4.50
Reagent H (Enz Sol)	-----	0.20

Incubate for 40 minutes at room temperature. Transfer the solutions to suitable cuvettes and record the A_{522nm} 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:

	<u>Std 1</u>	<u>Std 2</u>	<u>Std 3</u>	<u>Std 4</u>	<u>Std 5</u>	<u>Std Blank</u>
Acetoin	0.020	0.040	0.080	0.120	0.160	-----
Milli-Q	0.380	0.360	0.320	0.280	0.240	0.400
Color Solution	4.50	4.50	4.50	4.50	4.50	4.50

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

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CALCULATIONS:

Standard Curve:

$$r A_{522nm} \text{ Standard} = A_{522nm} \text{ Standard} - A_{522nm} \text{ Standard Blank}$$

Prepare a standard curve by plotting the $r A_{522nm}$ of the Standard versus μ moles of acetoin.

Sample Determination:

$$r A_{522nm} \text{ Test} = A_{522nm} \text{ Test} - A_{522nm} \text{ Test Blank}$$

Determine the micromoles of acetoin produced using the standard curve.

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

4.9 = Total volume (in milliliters) of assay

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 μ 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:

Stormer, F.C. (1975) *Methods in Enzymology*, XLI, 526-529

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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.