**Enzymatic Assay of (S)-DIACETYL REDUCTASE**

**PRINCIPLE:**

\[
\text{Diacetyl} + \beta\text{-NADPH} \xrightarrow{\text{(S)-Diacetyl Reductase}} \beta\text{-NADP} + (S)\text{-Acetoin}
\]

Abbreviations used:
- \(\beta\text{-NADPH}\) = \(\beta\text{-Nicotinamide Adenine Dinucleotide Phosphate, Reduced Form}\)
- \(\beta\text{-NADP}\) = \(\beta\text{-Nicotinamide Adenine Dinucleotide Phosphate, Oxidized Form}\)

**CONDITIONS:** \(T = 25^\circ C, \text{pH} = 6.9, A_{340nm}, \text{Light path} = 1 \text{ cm}\)

**METHOD:** Continuous Spectrophotometric Rate Determination

**REAGENTS:**

A. 100 mM Potassium Phosphate Buffer, pH 6.9 at 25ºC
   (Prepare 100 ml in deionized water using Potassium Phosphate, Monobasic, Anhydrous, Sigma Prod. No. P-5379. Adjust to pH 6.9 at 25ºC with 1 M KOH.)

B. 1 mM \(\text{DL-Dithiothreitol Solution (DTT)}\)
   (Prepare 25 ml in Reagent A using \(\text{DL-Dithiothreitol, Sigma Prod. No. D-0632.}\))

C. 114 mM Diacetyl Solution (Diacetyl)
   (Prepare 10 ml in deionized water using Diacetyl, Sigma Prod. No. D-3634.)

D. 1.10 mM \(\beta\text{-Nicotinamide Adenine Dinucleotide Phosphate, Reduced Form Solution (\(\beta\text{-NADPH}\)}\)
   (Dissolve the contents of one 1 mg vial of \(\beta\text{-Nicotinamide Adenine Dinucleotide Phosphate, Tetrasodium Salt, Preweighed Vial, Sigma Stock No. 201-201 in the appropriate volume of deionized water.})

E. (S)-Diacetyl Reductase Enzyme Solution
   (Immediately before use, prepare a solution containing 0.05 - 0.10 unit/ml of (S)-Diacetyl Reductase in cold Reagent A.)
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**PROCEDURE:**

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

<table>
<thead>
<tr>
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<th>Test</th>
<th>Blank</th>
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<tbody>
<tr>
<td>Reagent B (DTT)</td>
<td>0.75</td>
<td>0.75</td>
</tr>
<tr>
<td>Reagent D (β-NADPH)</td>
<td>0.10</td>
<td>0.10</td>
</tr>
<tr>
<td>Reagent E (Enzyme Solution)</td>
<td>0.10</td>
<td>0.10</td>
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</table>

Mix by inversion and equilibrate to 25°C. Monitor the $A_{340nm}$ until constant using a suitably thermostatted spectrophotometer. Then add:

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<tbody>
<tr>
<td>Reagent C (Diacetyl)</td>
<td>0.10</td>
<td>------</td>
</tr>
<tr>
<td>Deionized Water</td>
<td>------</td>
<td>0.10</td>
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</tbody>
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Immediately mix by inversion and record the decrease in $A_{340nm}$ for approximately 5 minutes. Obtain the $r A_{340nm}$/min using the maximum linear rate for both the Test and Blank.

**CALCULATIONS:**

$$\text{Units/ml enzyme} = \frac{(r A_{340nm}$/min Test - $r A_{340nm}$/min Blank)(1.05)(df)}{(6.22)(0.1)}$$

1.05 = 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 in assay

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

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

**UNIT DEFINITION:**

One unit will convert 1 µmole of diacetyl and β-NADPH to (S)-acetoin and β-NADP per minute at pH 6.9 at 25°C.
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FINAL ASSAY CONCENTRATIONS:

In a 1.05 ml reaction mix, the final concentrations are 81 mM potassium phosphate, 0.7 mM DL-dithiothreitol, 1.1 mM β-nicotinamide adenine dinucleotide phosphate, reduced form, 11 mM diacetyl, and 0.005 - 0.01 unit (S)-diacetyl reductase.

REFERENCE:


NOTES:

1. This assay is based on the cited reference.

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