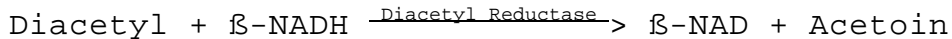


**Enzymatic Assay of DIACETYL REDUCTASE
(EC 1.1.1.5)**

PRINCIPLE:



Abbreviations used:

β -NADH = β -Nicotinamide Adenine Dinucleotide, Reduced Form

β -NAD = β -Nicotinamide Adenine Dinucleotide, Oxidized Form

CONDITIONS: T = 25°, pH 6.1, A_{340nm}, Light path = 1 cm

METHOD: Continuous Spectrophotometric Rate Determination

REAGENTS:

- A. 100 mM Potassium Phosphate Buffer, pH 6.1 at 25°C
(Prepare 100 ml in deionized water using Potassium Phosphate, Dibasic, Trihydrate, Sigma Prod. No. P-5504. Adjust to pH 6.1 at 25°C with 1 M HCl.)
- B. 0.25 mM β -Nicotinamide Adenine Dinucleotide, Reduced Form Solution (β -NADH)
(Dissolve the contents of one 1 mg vial of β -Nicotinamide Adenine Dinucleotide, Reduced Form, Disodium Salt, Preweighed Vial, Sigma Stock No. 340-101, in the appropriate volume of Reagent A.)
- C. 5% (w/v) Diacetyl Solution (Diacetyl)
(Prepare 1 ml in deionized water using Diacetyl, Sigma Prod. No. D-3634.)
- D. Diacetyl Reductase Enzyme Solution
(Immediately before use, prepare a solution containing 0.3 - 0.6 unit/ml of Diacetyl Reductase in cold Reagent A.)

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PROCEDURE:

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

	<u>Test</u>	<u>Blank</u>
Reagent B (β -NADH)	0.98	0.98

Incubate at 25°C for 5 minutes. Then add:

Reagent D (Enzyme Solution)	0.01	-----
Reagent A (Buffer)	-----	0.01

Mix by inversion and monitor the $A_{340\text{nm}}$ until constant using a suitably thermostatted spectrophotometer. Then add:

Reagent C (Diacetyl)	0.01	0.01
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Immediately mix by inversion and record the decrease in $A_{340\text{nm}}$ for approximately 5 minutes. Obtain the $r A_{340\text{nm}}$ /minute using the maximum linear rate for both the Test and Blank.

CALCULATIONS:

$$\text{Units/ml enzyme} = \frac{(r A_{340\text{nm}}/\text{min Test} - r A_{340\text{nm}}/\text{min Blank})(1)(\text{df})}{(6.22)(0.01)}$$

1 = Total volume (in milliliter) of assay

df = Dilution factor

6.22 = Millimolar extinction coefficient of β -NADH
at 340nm

0.01 = Volume (in milliliter) of enzyme used

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

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

UNIT DEFINITION:

One unit will oxidize 1 μ mole of NADH per minute at pH 6.1 at 25°C with diacetyl as substrate.

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FINAL ASSAY CONCENTRATIONS:

In a 1.00 ml reaction mix, the final concentrations are 99 mM potassium phosphate, 0.25 mM β -nicotinamide adenine dinucleotide, reduced form, 0.05% (w/v) diacetyl, and 0.003 - 0.006 unit diacetyl reductase.

REFERENCE:

Gibson, T.D., Parker, S.M., and Woodward, J.R. (1991)
Enzyme Microb. Technol. **13**, 171-178

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.