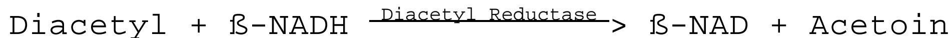
Enzymatic Assay of DIACETYL REDUCTASE
(EC 1.1.1.5)

PRINCIPLE:



Abbreviations used:
ß-NADH = ß-Nicotinamide Adenine Dinucleotide, Reduced Form
ß-NAD = ß-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 ß-Nicotinamide Adenine Dinucleotide, Reduced Form Solution (ß-NADH)
(Dissolve the contents of one 1 mg vial of ß-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.)

Enzymatic Assay of DIACETYL REDUCTASE
(EC 1.1.1.5)

PROCEDURE:

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

	Test	Blank
Reagent B (ß-NADH)	0.98	0.98

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

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

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

	Test	Blank
Reagent C (Diacetyl)	0.01	0.01

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

CALCULATIONS:

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

1 = Total volume (in milliliter) of assay
df = Dilution factor
6.22 = Millimolar extinction coefficient of ß-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 µmole of NADH per minute at pH 6.1 at 25°C with diacetyl as substrate.

Enzymatic Assay of DIACETYL REDUCTASE
(EC 1.1.1.5)

FINAL ASSAY CONCENTRATIONS:

In a 1.00 ml reaction mix, the final concentrations are 99 mM potassium phosphate, 0.25 mM ß-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.