Enzymatic Assay of (S)-DIACETYL REDUCTASE

PRINCIPLE:

Diacetyl + ß-NADPH $\xrightarrow{\text{(S)-Diacetyl Reductase}}$ ß-NADP + (S)-Acetoin

Abbreviations used:
ß-NADPH = ß-Nicotinamide Adenine Dinucleotide Phosphate, Reduced Form
ß-NADP = ß-Nicotinamide Adenine Dinucleotide Phosphate, Oxidized Form

CONDITIONS: T = 25°C, pH = 6.9, A_{340nm}, Light path = 1 cm

METHOD: Continuous Spectrophotometric Rate Determination

REAGENTS:

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

B. 1 mM DL-Dithiothreitol Solution (DTT)
(Prepare 25 ml in Reagent A using 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 ß-Nicotinamide Adenine Dinucleotide Phosphate, Reduced Form Solution (ß-NADPH)
(Dissolve the contents of one 1 mg vial of ß-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:

	Test	Blank
Reagent B (DTT)	0.75	0.75
Reagent D (ß-NADPH)	0.10	0.10
Reagent E (Enzyme Solution)	0.10	0.10

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

Reagent C (Diacetyl)	0.10	------
Deionized Water	------	0.10

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

CALCULATIONS:

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

1.05 = Total volume (in milliliters) of assay
df = Dilution factor
6.22 Millimolar extinction coefficient of ß-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 ß-NADPH to (S)-acetoin and ß-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 ß-nicotinamide adenine dinucleotide phosphate, reduced form, 11 mM diacetyl, and 0.005 - 0.01 unit (S)-diacetyl reductase.

REFERENCE:

Heidlas, J. and Tressl, R. (1990) *European Journal of Biochemistry* **188**, 165-174

NOTES:

1. This assay is based on the cited reference.