Enzymatic Assay of OCTOPINE DEHYDROGENASE (EC 1.5.1.11)

PRINCIPLE:

Pyruvate + L-Arginine + ß-NADH $\xrightarrow{\text{Octopine Dehydrogenase}}$ Octopine + H_2O + ß-NAD

Abbreviations:
ß-NADH = ß-Nicotinamide Adenine Dinucleotide, Reduced Form
ß-NAD = ß-Nicotinamide Adenine Dinucleotide, Oxidized Form

CONDITIONS: T = 25°C, pH = 6.6, A_{340nm}, Light path = 1 cm

METHOD: Continuous Spectrophotometric Rate Determination

REAGENTS:

A. 150 mM Sodium Phosphate Buffer, pH 6.6 at 25°C
(Prepare 200 ml in deionized water using Sodium Phosphate, Monobasic, Anhydrous, Sigma Prod. No. S-0751. Adjust to pH 6.6 at 25°C with 1 M NaOH.)

B. 60 mM Sodium Pyruvate Solution (Pyruv)
(Prepare 10.0 ml in deionized water using Pyruvic Acid, Sodium Salt, Sigma Prod. No. P-2256. **PREPARE FRESH**.)

C. 60 mM L-Arginine Solution (Arg)
(Prepare 5 ml in deionized water using L-Arginine Free Base, Sigma Prod. No. A-5006.)

D. 4.2 mM ß-Nicotinamide Adenine Dinucleotide, Reduced Form Solution (ß-NADH)
(Dissolve the contents of one 5 mg vial of ß-Nicotinamide Adenine Dinucleotide, Reduced Form, Disodium Salt, Sigma Stock No. 340-105, in the appropriate volume of cold Reagent A. **PREPARE FRESH.)**

E. Octopine Dehydrogenase Enzyme Solution
(Immediately before use, prepare a solution containing 0.25 - 0.50 unit/ml of Octopine Dehydrogenase in cold Reagent A.)

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PROCEDURE:

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

	Test	Blank
Reagent A (Buffer)	2.45	2.50
Reagent B (Pyruv)	0.20	0.20
Reagent C (Arg)	0.20	0.20
Reagent D (ß-NADH)	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 E (Enzyme Solution)	0.05	------

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

CALCULATIONS:

$$\text{Units/mg enzyme} = \frac{(\Delta A_{340nm}/\text{min Test} - \Delta A_{340nm}/\text{min Blank})(3)(df)}{(6.22)(0.05)}$$

3 = Total volume (in milliliters) of assay
df = Dilution factor
6.22 = Millimolar extinction coefficient of ß-NADH at 340 nm
0.05 = 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 form 1.0 µmole of octopine from 1.0 µmole of pyruvate and 1.0 µmole of L-arginine per minute at pH 6.6 at 25°C.

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FINAL ASSAY CONCENTRATION:

In a 3.00 ml reaction mix, the final concentrations are 130 mM sodium phosphate, 4 mM sodium pyruvate, 4 mM L-arginine, 0.14 mM ß-nicotinamide adenine dinucleotide, reduced form and 0.0125 - 0.025 unit octopine dehydrogenase.

REFERENCE:

Gaede, G. and Grieshaber, M. (1975) *Analytical Biochemistry* **66**, 393-399

NOTES:

1. This assay is based on the cited reference.

3. Where Sigma Product or Stock numbers are specified, equivalent reagents may be substituted.