

**Enzymatic Assay of OCTOPINE DEHYDROGENASE
(EC 1.5.1.11)**

PRINCIPLE:

Pyruvate + L-Arginine + β -NADH $\xrightarrow{\text{Octopine Dehydrogenase}}$ Octopine + H₂O + β -NAD

Abbreviations:

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

β -NAD = β -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 β -Nicotinamide Adenine Dinucleotide, Reduced Form Solution (β -NADH)
(Dissolve the contents of one 5 mg vial of β -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:

	<u>Test</u>	<u>Blank</u>
Reagent A (Buffer)	2.45	2.50
Reagent B (Pyruv)	0.20	0.20
Reagent C (Arg)	0.20	0.20
Reagent D (β-NADH)	0.10	0.10

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

Reagent E (Enzyme Solution)	0.05	-----
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Immediately mix by inversion and record the decrease in $A_{340\text{nm}}$ for approximately 5 minutes. Obtain the $r A_{340\text{nm}}/\text{minute}$ using the maximum linear rate for both the Test and Blank.

CALCULATIONS:

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

3 = Total volume (in milliliters) of assay

df = Dilution factor

6.22 = Millimolar extinction coefficient of β-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 β -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.

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