Enzymatic Assay of FORMALDEHYDE DEHYDROGENASE (EC 1.2.1.46)

PRINCIPLE:

Formaldehyde + ß-NAD $\xrightarrow{FDH}$ Formate + ß-NADH

Abbreviations used:
ß-NAD = ß-Nicotinamide Adenine Dinucleotide, Oxidized Form
ß-NADH = ß-Nicotinamide Adenine Dinucleotide, Reduced Form
FDH = Formaldehyde Dehydrogenase

CONDITIONS: T = 37°C, pH = 7.5, A_{340nm}, Light path = 1 cm

METHOD: Continuous Spectrophotometric Rate Determination

REAGENTS:

A. 50 mM Potassium Phosphate Buffer, pH 7.5 at 37°C
(Prepare 50 ml in deionized water using Potassium Phosphate, Monobasic, Anhydrous, Sigma Prod. No. P-5379. Adjust to pH 7.5 at 37°C with 1 M KOH.)

B. 5.7 mM ß-Nicotinamide Adenine Dinucleotide Solution (ß-NAD)
(Prepare 5 ml in deionized water using ß-Nicotinamide Adenine Dinucleotide, Sigma Prod. No. N-7004 or dissolve the contents of one 20 mg vial of ß-Nicotinamide Adenine Dinucleotide, Sigma Stock No. 260-120, in the appropriate volume of deionized water. **PREPARE FRESH.**)

C. 0.08% (v/v) Formaldehyde Solution (Formaldehyde)
(Prepare by adding 0.1 ml of Formaldehyde, 37% (w/w) Solution (Formalin), Sigma Prod. No. F-1635, to 45 ml of deionized water.)

D. Formaldehyde Dehydrogenase Enzyme Solution
(Immediately before use, prepare a solution containing 0.5 - 1.0 unit/ml of Formaldehyde Dehydrogenase in cold Reagent A.)

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PROCEDURE:

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

	Test	Blank
Deionized Water	0.30	0.30
Reagent A (Buffer)	2.00	2.05
Reagent B (ß-NAD)	0.50	0.50
Reagent C (Formaldehyde)	0.10	0.10

Mix by inversion and equilibrate to 37°C. Monitor the A_{340nm} until constant using a suitably thermostatted spectrophotometer. Then add:

Reagent D (Enzyme Solution)	0.05	------

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

CALCULATIONS:

$$\text{Units/ml enzyme} = \frac{(\Delta A_{340nm}/\text{min Test} - \Delta A_{340nm}/\text{min Blank})(2.95)(df)}{(6.22)(0.05)}$$

2.95 = 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

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

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

UNIT DEFINITION:

One unit will oxidize 1.0 µmole of formaldehyde to formic acid per minute at pH 7.5 at 37°C.

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FINAL ASSAY CONCENTRATION:

In a 2.95 ml reaction mix, the final concentrations are 35 mM potassium phosphate, 1.0 mM ß-nicotinamide adenine dinucleotide, 0.003% (v/v) formaldehyde, and 0.03 - 0.05 unit formaldehyde dehydrogenase.

REFERENCES:

Ando, M., Yoshimoto, T., Ogushi, S., Rikitake, K., Shibata, S., and Tsuru, D. (1979) *Journal of Biochemistry* **82**, 1165-1172

NOTES:

1. This assay is based on the cited reference.

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