Enzymatic Assay of FORMALDEHYDE DEHYDROGENASE (EC 1.2.1.1)

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. 6.0 mM ß-Nicotinamide Adenine Dinucleotide Solution (ß-NAD)
(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% Solution, Sigma Prod. No. F-1635, to 45 ml of deionized water.)

D. 60 mM Reduced Glutathione Solution (Glutathione)
(Prepare 2 ml in deionized water using Glutathione, Reduced Form, Free Acid, Sigma Prod. No. G-4251.)

E. Formaldehyde Dehydrogenase Enzyme Solution
(Immediately before use, prepare a solution containing 0.25 - 0.50 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	0.20	0.20
Reagent A (Buffer)	2.00	2.10
Reagent B (ß-NAD)	0.50	0.50
Reagent C (Formaldehyde)	0.10	0.10
Reagent D (Glutathione)		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 E (Enzyme Solution)	0.10	------

Immediately mix by inversion and record the increase 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:

$$\text{Units/mg solid} = \frac{r\ A_{340nm}/\text{min Test} - r\ A_{340nm}/\text{min Blank}}{(6.22)\ (\text{mg solid/ml RM})}$$

6.22 = Millimolar extinction coefficient of ß-NADH at 340 nm
RM = Reaction Mix

UNIT DEFINITION:

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

FINAL ASSAY CONCENTRATION:

In a 3.00 ml reaction mix, the final concentrations are 35 mM potassium phosphate, 1.0 mM ß-NAD, 2.0 mM glutathione,
0.0027% (v/v) formaldehyde and 0.025 - 0.050 unit formaldehyde dehydrogenase.

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REFERENCE:

Rose, Z. B. and Racker, E. (1966) *Methods in Enzymology*, Volume IX, 357-360.

NOTES:

1. This assay is a modification of the assay described in the cited reference.

2. All product and stock numbers, unless otherwise indicated, are Sigma product and stock numbers.