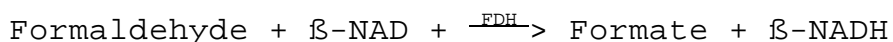


**Enzymatic Assay of FORMALDEHYDE DEHYDROGENASE
(EC 1.2.1.1)**

PRINCIPLE:



Abbreviations used:

β -NAD = β -Nicotinamide Adenine Dinucleotide, Oxidized Form

β -NADH = β -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 β -Nicotinamide Adenine Dinucleotide Solution (β -NAD)
(Dissolve the contents of one 20 mg vial of β -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:

| | <u>Test</u> | <u>Blank</u> |
|--------------------------|-------------|--------------|
| Deionized Water | 0.20 | 0.20 |
| Reagent A (Buffer) | 2.00 | 2.10 |
| Reagent B (β-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 $A_{340\text{nm}}$ until constant, using a suitably thermostatted spectrophotometer. Then add:

| | | |
|-----------------------------|------|-------|
| Reagent E (Enzyme Solution) | 0.10 | ----- |
|-----------------------------|------|-------|

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

CALCULATIONS:

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

6.22 = Millimolar extinction coefficient of β-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 β-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.

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