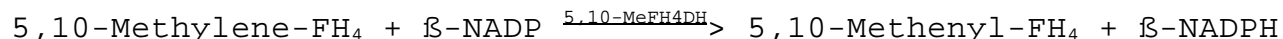


**Enzymatic Assay of 5,10-METHYLENETETRAHYDROFOLATE
DEHYDROGENASE
(EC 1.5.1.5)**

PRINCIPLE:



Abbreviations used:

5,10-Methylene-FH₄ = 5,10-Methylenetetrahydrofolate

5,10-Methenyl-FH₄ = 5,10-Methenyltetrahydrofolate

5,10-MeFH₄DH = 5,10-Methylenetetrahydrofolate Dehydrogenase

β-NADP = β-Nicotinamide Adenine Dinucleotide Phosphate,
Oxidized Form

β-NADPH = β-Nicotinamide Adenine Dinucleotide Phosphate,
Reduced Form

CONDITIONS: T = 25°C, pH = 7.5, A_{340nm}, Light path = 1 cm

METHOD: Continuous Spectrophotometric Rate Determination

REAGENTS:

- A. 50 mM Potassium Phosphate Buffer with 100 mM Potassium Chloride, pH 7.5 at 25°C
(Prepare 200 ml in deionized water using Potassium Phosphate, Monobasic, Anhydrous, Sigma Prod. No. P-5379, and Potassium Chloride, Sigma Prod. No. P-4504. Adjust to pH 7.5 at 25°C with 1 M NaOH.)
- B. 0.002% (w/v) Tetrahydrofolic Acid with 0.002% (v/v) Formaldehyde and 0.1% (v/v) 2-Mercaptoethanol Solution (FH₄)
(Immediately before used, prepare 100 ml in Reagent A using Tetrahydrofolic Acid, Sigma Prod. No. T-3125, Formaldehyde, 37% Solution, Sigma Prod. No. F-1635, and 2-Mercaptoethanol, Sigma Prod. No. M-6250.¹)
- C. 20 mM β-Nicotinamide Adenine Dinucleotide Phosphate (β-NADP)
(Prepare 2 ml in deionized water using β-Nicotinamide Adenine Dinucleotide Phosphate, Sodium Salt, Sigma Prod. No. N-0505. **PREPARE FRESH.**)

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REAGENTS: (continued)

- D. 5,10-Methylenetetrahydrofolate Dehydrogenase Enzyme Solution
(Immediately before use, prepare a solution containing 0.01 - 0.03 unit/ml of 5,10-Methylenetetrahydrofolate Dehydrogenase in cold Reagent A.)

PROCEDURE:

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

	Test	Blank
Reagent B (FH ₄)	2.80	2.80
Reagent D (Enzyme Solution)	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 C (β-NADP)	0.10	-----
Deionized Water	-----	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:

$$\text{Units/mg enzyme} = \frac{r_{A_{340\text{nm}}/\text{min Test}} - r_{A_{340\text{nm}}/\text{min Blank}}}{(7.1) (\text{mg enzyme/ml RM})}$$

7.1 = Millimolar extinction coefficient of β-NADPH at 340 nm

under the conditions of the assay.²

RM = Reaction Mix

UNIT DEFINITION:

One unit will convert 1.0 μmole 5,10-methylenetetrahydrofolate and NADP to 5,10-methenyltetrahydrofolate and NADPH per minute at pH 7.5 at 25°C.

**Enzymatic Assay of 5,10-METHYLENETETRAHYDROFOLATE
DEHYDROGENASE**

(EC 1.5.1.5)

FINAL ASSAY CONCENTRATION:

In a 3.00 ml reaction mix, the final concentrations are 48 mM potassium phosphate, 97 mM potassium chloride, 0.002% (v/v) formaldehyde, 0.09% (v/v) 2-mercaptoethanol, 0.002% (w/v) tetrahydrofolic acid, 0.67 mM β -NADP and 0.001 - 0.003 unit/ml 5,10-methylenetetrahydrofolate dehydrogenase.

REFERENCE:

Ramasastri, B.V. and Blakley, R.L. (1964) *Journal of Biological Chemistry* **239**, 112-114.

NOTES:

1. Weigh the Tetrahydrofolate in the dark. Allow the solution to stand at room temperature, protected from light, for 5 minutes to generate 5,10-Methylene-tetrahydrofolic acid.
2. The millimolar extinction coefficient of NADP under the conditions of this assay is 7.1 as per the cited reference.
3. All products 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.