Enzymatic Assay of DIHYDROFOLATE REDUCTASE (EC 1.5.1.3)

PRINCIPLE:

DHFA + ß-NADPH $\xrightarrow{\text{Dihydrofolate Reductase}}$ THFA + ß-NADP

Abbreviations used:
DHFA = 7,8-Dihydrofolic acid
THFA = 5,6,7,8-Tetrahydrofolic Acid
ß-NADPH = ß-Nicotinamide Adenine Dinucleotide Phosphate, Reduced Form
ß-NADP = ß-Nicotinamide Adenine Dinucleotide Phosphate, Oxidized Form

CONDITIONS: T = 25°C, pH = 6.5, A_{340nm}, Light Path = 1 cm

METHOD: Continuous Spectrophotometric Rate Determination

REAGENTS:

A. 50 mM Potassium Phosphate Buffer, pH 6.5 at 25°C
(Prepare 100 ml in deionized water using Potassium Phosphate, Monobasic, Anhydrous, Sigma Prod. No. P-5379. Adjust to pH 6.5 at 25°C with 1 M KOH.)

B. 0.11 mM ß-Nicotinamide Adenine Dinucleotide Phosphate, Reduced Form Solution (ß-NADPH)
(Dissolve the contents of one 5 mg vial of ß-Nicotinamide Adenine Dinucleotide Phosphate, Reduced Form, Tetrasodium Salt, Sigma Stock No. 201-205, in the appropriate volume of Reagent A. **PREPARE FRESH**.)

C. 2.3 mM Dihydrofolic Acid Solution (DHFA)
(Immediately before use, prepare 1 ml in Reagent A using Dihydrofolic Acid, Sigma Prod. No. D-7006. Facilitate dissolution with 1 M KOH (0.020 ml of 1 M KOH per mg of Dihydrofolic Acid). **PREPARE FRESH. This solution is stable for only 10 minutes**.)

D. 0.1% (w/v) Bovine Serum Albumin Solution (BSA)
(Prepare 100 ml in Reagent A using Albumin, Bovine, Sigma Prod. No. A-4503.)

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REAGENTS: (continued)

E. Dihydrofolate Reductase Enzyme Solution
(Immediately before use, prepare a solution containing 0.12 - 0.25 unit/ml of Dihydrofolate Reductase in cold Reagent D.)

PROCEDURE:

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

	Test		Blank
Reagent B (ß-NADPH)	3.00		3.00
Reagent C (DHFA)	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 E (Enzyme Solution)	0.10	------
Reagent D (BSA)	------	0.10

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

CALCULATIONS:

$$\text{Units/ml enzyme} = \frac{(\Delta A_{340nm}/\text{min Test} - \Delta A_{340nm}/\text{min Blank})(3.2)(df)}{(12.3)(0.1)}$$

3.2 = Total volume (in milliliters) of assay
df = Dilution factor
12.3 = Difference in the millimolar extinction coefficients of the substrates and the products in the dihydrofolate reductase reaction
0.1 = Volume (in milliliters) of assay

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

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

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UNIT DEFINITION:

One unit will convert 1.0 µmole of 7,8-dihydrofolate and ß-NADPH to 5,6,7,8-tetrahydrofolate and ß-NADP per minute at pH 6.5 at 25°C.

FINAL ASSAY CONCENTRATION:

In a 3.20 ml reaction mix, the final concentrations are 50 mM potassium phosphate, 0.072 mM dihydrofolic acid, 0.10 mM ß-nicotinamide adenine dinucleotide phosphate, reduced form, 0.003% (w/v) bovine serum albumin and 0.012 - 0.025 unit dihydrofolate reductase.

REFERENCES:

Hillcoat, B.L., Nixon, P.F., and Blakely, R.F (1967) *Anal. Biochem.* **21**, 178-189.

Peterson, D.L., Gleisner, J.M., and Blakley, R.L. (1975) *Biochemistry* **14**, 5261-5267.

NOTES:

1. The difference between the millimolar extinction coefficients is described in Hillcoat, B.L. et al. (1967).

2. This assay is based on the cited references.

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.