Enzymatic Assay of DIHYDROPTERIDINE REDUCTASE
(EC 1.6.99.7)

PRINCIPLE:

6,7-DMTHP + H_2O_2 $\xrightarrow{\text{Peroxidase}}$ 6,7-DMDHP (Quinonoid Isomer)

6,7-DMDHP (Quinonoid Isomer) + ß-NADH $\xrightarrow{\text{DHPR}}$ 6,7-DMTHP + ß-NAD

Abbreviations used:
DHPR = Dihydropteridine Reductase
6,7-DMTHP = 6,7-Dimethyltetrahydropterine
6,7-DMDHP = 6,7-Dimethyldihydropterine
ß-NADH = ß-Nicotinamide Adenine Dinucleotide, Reduced Form
ß-NAD = ß-Nicotinamide Adenine Dinucleotide, Oxidized Form

CONDITIONS: T = 25°C, pH = 7.2, A_{340nm}, Light Path = 1 cm

METHOD: Continuous Spectrophotometric Rate Determination

REAGENTS:

A. 100 mM Tris HCl Buffer, pH 7.2 at 25°C
(Prepare 100 ml in deionized water using Trizma Base, Sigma Prod. No. T-1503. Adjust to pH 7.2 at 25°C with 1 M HCl.)

B. 0.43 mM ß-Nicotinamide Adenine Dinucleotide, Reduced Form Solution (ß-NADH)
(Dissolve the contents of one 1 mg vial of ß-Nicotinamide Adenine Dinucleotide, Reduced Form, Disodium Salt, Sigma Stock No. 340-101, in the appropriate volume of deionized water. **PREPARE FRESH.**)

C. 0.3% (v/v) Hydrogen Peroxide Solution (H_2O_2)
(Prepare 10 ml in deionized water using Hydrogen Peroxide, 30% (w/w), Sigma Prod. No. H-1009.)

REAGENTS: (Continued)

D. 2.15 mM 6,7-Dimethyltetrahydropterine Solution (6,7-DMTHP)
(Immediately before use prepare 2 ml in cold Reagent A using 6,7-Dimethyl-5,6,7,8-Tetrahydropterine, Monohydrochloride, Sigma Prod. No. D-0387.)

E. Peroxidase Enzyme Solution
(Immediately before use prepare a solution containing 100 purpurogallin units/ml of Peroxidase, Sigma Prod. No. P-8250 in cold deionized water.)

F. Dihydropteridine Reductase Enzyme Solution (DHPR)
(Immediately before use prepare a solution containing 0.08 - 0.32 unit/ml using cold Reagent A.)

PROCEDURE:

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

	Test	Blank
Reagent A (Buffer)	1.50	1.50
Reagent C (H_2O_2)	0.03	0.03
Reagent D (6,7-DMTHP)	0.05	0.05
Reagent B (ß-NADH)	1.00	1.00
Reagent E (Peroxidase Enzyme Solution)	0.05	0.05
Deionized Water	0.27	0.27

Mix by inversion and equilibrate to 25°C. Monitor the A_{340nm} until constant, using a suitably thermostatted spectrophotometer. Then add:

	Test	Blank
Reagent F (DHPR)	0.10	-----
Reagent A (Buffer)	------	0.10

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

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CALCULATIONS:

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

6.22 = Millimolar extinction coefficient of ß-NADH at 340 nm[1]
RM = Reaction Mix

UNIT DEFINITION:

One unit will oxidize 1.0 µmole of ß-NADH to ß-NAD with 6,7-Dimethyldihydropterine (quinonoid isomer) as the non-nucleotide substrate per minute at pH 7.2 at 25°C.

FINAL ASSAY CONCENTRATION:

In a 3.00 ml reaction mix, the final concentrations are 55 mM Tris, 0.14 mM ß-NADH, 0.003% (w/w) hydrogen peroxide,
0.04 mM 6,7-dimethyl-5,6,7,8-tetrahydropterine,
5 units peroxidase, and 0.008-0.032 unit dihydropteridine reductase.

REFERENCES:

Craine, J.E. et al., (1972) *Journal of Biological Chemistry* **247**, 6082-6091.

Webber, S. et al., (1978) *Analytical Biochemistry* **84**, 491-503.

NOTES:

1. Sigma uses 6.22 as the millimolar extinction coefficient although Webber (reference 2) suggests using 10.7 which includes the contribution from the non-nucleotide substrate.

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.