

**Enzymatic Assay of DIHYDROPTERIDINE REDUCTASE  
(EC 1.6.99.7)**

**PRINCIPLE:**

6,7-DMTHP + H<sub>2</sub>O<sub>2</sub>  $\xrightarrow{\text{Peroxidase}}$  6,7-DMDHP (Quinonoid Isomer)

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

Abbreviations used:

DHPR = Dihydropteridine Reductase

6,7-DMTHP = 6,7-Dimethyltetrahydropterine

6,7-DMDHP = 6,7-Dimethyldihydropterine

$\beta$ -NADH =  $\beta$ -Nicotinamide Adenine Dinucleotide, Reduced Form

$\beta$ -NAD =  $\beta$ -Nicotinamide Adenine Dinucleotide, Oxidized Form

**CONDITIONS:** T = 25°C, pH = 7.2, A<sub>340nm</sub>, 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  $\beta$ -Nicotinamide Adenine Dinucleotide, Reduced Form Solution ( $\beta$ -NADH)  
(Dissolve the contents of one 1 mg vial of  $\beta$ -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<sub>2</sub>O<sub>2</sub>)  
(Prepare 10 ml in deionized water using Hydrogen Peroxide, 30% (w/w), Sigma Prod. No. H-1009.)

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**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:

	<u>Test</u>	<u>Blank</u>
Reagent A (Buffer)	1.50	1.50
Reagent C (H <sub>2</sub> O <sub>2</sub> )	0.03	0.03
Reagent D (6,7-DMTHP)	0.05	0.05
Reagent B (β-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<sub>340nm</sub> until constant, using a suitably thermostatted spectrophotometer. Then add:

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

Immediately mix by inversion and record the decrease in A<sub>340nm</sub> for approximately 5 minutes. Obtain the ΔA<sub>340nm</sub>/minute using the maximum linear rate for both the Test and Blank.

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**CALCULATIONS:**

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

6.22 = Millimolar extinction coefficient of  $\beta$ -NADH  
at 340 nm<sup>1</sup>

RM = Reaction Mix

**UNIT DEFINITION:**

One unit will oxidize 1.0  $\mu$ mole of  $\beta$ -NADH to  $\beta$ -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  $\beta$ -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.**