Enzymatic Assay of TYROSINE HYDROXYLASE
(EC 1.14.16.2)

PRINCIPLE:

$_L$-Tyrosine + DMTHP + O_2 $\xrightarrow{TH}$ $_L$-DOPA + Quinonoid Dihydropterin + H_2O

Quinonoid Dihydropterin + ß-NADH $\xrightarrow{DHT\text{-}RDase}$ ß-NAD + DMTHP

Abbreviations used:
DMTHP = 6,7-Dimethyl-5,6,7,8-Tetrahydropterine
TH = Tyrosine Hydroxylase
$_L$-DOPA = $_L$-3,4-Dihydroxyphenylalanine
ß-NADH = ß-Nicotinamide Adenine Dinucleotide, Reduced Form
DHT-RDase = Dihydropteridine Reductase
ß-NAD = ß-Nicotinamide Adenine Dinucleotide, Oxidized Form

CONDITIONS: T = 37°C, pH = 7.0, A_{340nm}, Light path = 1 cm

METHOD: Continuous Spectrophotometric Rate Determination

REAGENTS:

A. 100 mM Tris HCl Buffer, pH 7.0 at 37°C (Buffer)
(Prepare 100 ml in deionized water using Trizma Base, Sigma Prod. No. T-1503. Adjust to pH 7.0 at 37°C with 1 M HCl.)

B. 30 mM $_L$-Tyrosine Solution, pH 7.0 at 37°C ($_L$-Tyr)
(Prepare 10 ml in deionized water using $_L$-Tyrosine, Sigma Prod. No. T-3754. Adjust to pH 7.0 at 37°C with 1 M NaOH.)

C. 10 mM 6,7-Dimethyl-5,6,7,8-Tetrahydropterine Solution (DMTHP)
(Immediately before use prepare 2 ml in deionized water using 6,7-Dimethyl-5,6,7,8-Tetrahydropterine, Monohydrochloride, Sigma Prod. No. D-0387.)

D. 0.43 mM ß-Nicotinamide Adenine Dinucleotide, Reduced Form, Solution (ß-NADH)
(Dissolve the contents of one 10 mg vial of ß-Nicotinamide Adenine Dinucleotide, Reduced Form, Disodium Salt, Sigma Stock No. 340-110, in the appropriate volume of deionized water. **PREPARE FRESH**.)

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REAGENTS: (continued)

E. Catalase[1] Enzyme Solution (Catalase)
(Immediately before use, prepare a solution containing 10,000 units/ml of Catalase, Sigma Stock No. C-100, in cold Reagent A.)

F. Dihydropteridine Reductase Enzyme Solution (DHT-RDase)
(Immediately before use, prepare a solution containing 5 units/ml of Dihyropteridine Reductase, Sigma Prod. No. D-6888, in cold Reagent A.)

G. Tyrosine Hydroxylase Enzyme Solution (TH)
(Immediately before use, prepare a solution containing 150 - 450 units/ml of Tyrosine Hydroxylase in cold Reagent A.)

PROCEDURE:

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

	Test	Blank
Reagent A (Buffer)	1.50	1.50
Reagent B (L-Tyr)	0.03	0.03
Reagent C (DMTHP)	0.05	0.05
Reagent D (ß-NADH)	1.00	1.00
Reagent E (Catalase)	0.05	0.05
Reagent F (DHT-RDase)	0.10	0.10
Deionized Water	0.17	0.20

Mix by inversion and equilibrate to 37°C. Monitor the A_{340nm} until constant, using a suitably thermostatted spectrophotometer. Then add:

Reagent G (TH)	0.03	------

Immediately mix by inversion and record the decrease in A_{340nm} for approximately 5 minutes. Obtain the $_{r}$ A_{340nm}/minute using the maximum linear rate for both the Test and Blank.[2]

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CALCULATIONS:

$$\text{Units/ml enzyme} = \frac{(\text{r } A_{340nm}/\text{min Test} - \text{r } A_{340nm}/\text{min Blank})(2.93)(df)(1000)}{(6.22)(0.03)}$$

2.93 = Total volume (in milliliters) of assay
df = Dilution factor
1000 = Conversion factor from µmoles to nanomoles as per the Unit Definition
6.22 = Millimolar extinction coefficient of ß-NADH at 340 nm
0.03 = Volume (in milliliter) of enzyme used

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

UNIT DEFINITION:

One unit will form 1.0 nanomole of L-DOPA from tyrosine per minute at pH 7.0 at 37°C.

FINAL ASSAY CONCENTRATIONS:

In a 2.93 ml reaction mix, the final concentrations are 57 mM Tris, 0.3 mM L-tyrosine, 0.2 mM 6,7-dimethyl-5,6,7,8-tetrahyropterine, 0.15 mM ß-nicotinamide adenine dinucleotide, reduced form, 500 units catalase, 0.5 unit dihydropteridine reductase, and 4.5 - 14 units tyrosine hydroxylase.

REFERENCE:

Craine, J.E., Hall, E.S., and Kaufman, S. (1972) *Journal of Biological Chemistry* **247**, 6082-6091

Shiman, R., Akino, M. and Kaufman, S. (1971) *Journal of Biological Chemistry* **246**, 1330-1340

NOTES:

1. Catalase is added to protect the assay system against peroxide-mediated inactivation as described in Shiman, R. et al.

2. A significant rate for the blank (A_{340nm}/min = 0.03 - 0.04) will occur.

NOTES: (continued)

3. Catalase Unit Definition: One unit will decompose 1.0 µmole of H_2O_2 per minute at pH 7.0 at 25°C, while the H_2O_2 concentration falls from 10.3 to 9.2 mM. The rate of disappearance of H_2O_2 is followed by observing the rate of decrease in absorbance at 240 nm.

4. Dihydropteridine Reductase 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.

5. 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.