Enzymatic Assay of TYROSINE HYDROXYLASE
(EC 1.14.16.2)

PRINCIPLE:

\[ \text{L-Tyrosine} + \text{DMTHP} + O_2 \xrightarrow{\text{TH}} \text{L-DOPA} + \text{Quinonoid Dihydropterin} + H_2O \]

\[ \text{Quinonoid Dihydropterin} + \beta-\text{NADH} \xrightarrow{\text{DHT-RDase}} \beta-\text{NAD} + \text{DMTHP} \]

Abbreviations used:

DMTHP = 6,7-Dimethyl-5,6,7,8-Tetrahydropterine
TH = Tyrosine Hydroxylase
L-DOPA = L-3,4-Dihydroxyphenylalanine
β-NADH = β-Nicotinamide Adenine Dinucleotide, Reduced Form
DHT-RDase = Dihydropteridine Reductase
β-NAD = β-Nicotinamide Adenine Dinucleotide, Oxidized Form

CONDITIONS:  \( T = 37^\circ C, \ \text{pH} = 7.0, A_{340\text{nm}}, \ \text{Light path} = 1 \ \text{cm} \)

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 β-Nicotinamide Adenine Dinucleotide, Reduced Form, Solution (β-NADH)  
(Dissolve the contents of one 10 mg vial of β-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:

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
e 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:

<table>
<thead>
<tr>
<th></th>
<th>Test</th>
<th>Blank</th>
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<tbody>
<tr>
<td>Reagent A (Buffer)</td>
<td>1.50</td>
<td>1.50</td>
</tr>
<tr>
<td>Reagent B (L-Tyr)</td>
<td>0.03</td>
<td>0.03</td>
</tr>
<tr>
<td>Reagent C (DMTHP)</td>
<td>0.05</td>
<td>0.05</td>
</tr>
<tr>
<td>Reagent D (ß-NADH)</td>
<td>1.00</td>
<td>1.00</td>
</tr>
<tr>
<td>Reagent E (Catalase)</td>
<td>0.05</td>
<td>0.05</td>
</tr>
<tr>
<td>Reagent F (DHT-RDase)</td>
<td>0.10</td>
<td>0.10</td>
</tr>
<tr>
<td>Deionized Water</td>
<td>0.17</td>
<td>0.20</td>
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</tbody>
</table>

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

<p>| | |</p>
<table>
<thead>
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<tbody>
<tr>
<td>Reagent G (TH)</td>
<td>0.03</td>
</tr>
</tbody>
</table>

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

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

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 β-NADH at 340 nm
0.03 = Volume (in milliliter) of enzyme used

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

UNIT DEFINITION:

One unit will form 1.0 nanomole of \( \text{L-DOPA} \) 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 \( \text{L-tyrosine} \), 0.2 mM 6,7-dimethyl-5,6,7,8-tetrahydropyrimidine, 0.15 mM β-nicotinamide adenine dinucleotide, reduced form, 500 units catalase, 0.5 unit dihydropteridine reductase, and 4.5 - 14 units tyrosine hydroxylase.

REFERENCE:


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 \( (A_{340\text{nm}}/\text{min} = 0.03 - 0.04) \) will occur.
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NOTES:  (continued)

3. Catalase Unit Definition:  One unit will decompose 1.0 µmole of H₂O₂ per minute at pH 7.0 at 25°C, while the H₂O₂ concentration falls from 10.3 to 9.2 mM. The rate of disappearance of H₂O₂ 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 β-NADH to β-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.