

**Enzymatic Assay of DOPAMINE β -HYDROXYLASE
(EC 1.14.17.1)**

PRINCIPLE:

Tyramine + Ascorbate + O₂ $\xrightarrow{\text{Dopamine } \beta\text{-Hydroxylase}}$ Octopamine

Octopamine + Periodate \longrightarrow p-Hydroxybenzaldehyde

CONDITIONS: T = 37°C, pH = 5.0, A_{330nm}, Light path = 1 cm

METHOD: Spectrophotometric Stopped Rate Determination

REAGENTS:

- A. 1 M Sodium Acetate Buffer, pH 5.0 at 37°C
(Prepare 100 ml in deionized water using Sodium Acetate, Trihydrate, Sigma Prod. No. S-8625. Adjust to pH 5.0 at 37°C with 1 M HCl.)
- B. 400 mM Tyramine Hydrochloride Solution
(Prepare 5 ml in deionized water using Tyramine Hydrochloride, Sigma Prod. No. T-2879.)
- C. 200 mM Sodium Ascorbate Solution (Asc)
(Prepare 10 ml in deionized water using L-Ascorbic Acid, Sodium Salt, Sigma Prod. No. A-7631. **PREPARE FRESH.**)
- D. 200 mM Sodium Fumarate Solution (Fum)
(Prepare 10 ml in deionized water using Fumaric Acid, Disodium Salt, Sigma Prod. No. F-1506. **PREPARE FRESH.**)
- E. 200 mM N-Ethylmaleimide Solution (NEM)
(Prepare 10 ml in deionized water using N-Ethylmaleimide, Sigma Prod. No. E-3876. **PREPARE FRESH.**)
- F. 94 mM Sodium m-Periodate Solution (Per)
(Prepare 10 ml in deionized water using Sodium m-Periodate, Sigma Prod. No. S-1878. **PREPARE FRESH.**)

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REAGENTS: (continued)

- G. 530 mM Sodium Bisulfite Solution (Bisul)
(Prepare 10 ml in deionized water using Sodium Bisulfite, Sigma Prod. No. S-9000. **PREPARE FRESH.**)
- H. 0.5 mM DL-Octopamine Standard Solution (Octo)
(Prepare 100 ml in deionized water using DL-Octopamine Hydrochloride, Sigma Prod. No. O-0250.)
- I. Catalase Enzyme Solution (Cat)
(Immediately before use, prepare a solution containing 35,000 units/ml in deionized water using Catalase, Sigma Prod. No. C-3515.)
- J. Dopamine β -Hydroxylase Enzyme Solution
(Immediately before use, prepare a solution containing 0.15 - 0.30 unit/ml of Dopamine β -Hydroxylase in cold deionized water.)
- K. Dowex 50W Ion Exchange Resin
(Prepare 5 ml using Dowex 50W Hydrogen Resin, Sigma Stock No. 50X4-400, by washing with deionized water until the eluate is clear and colorless.)
- L. 3 M Trichloroacetic Acid Solution (TCA)
(Prepare 5 ml in deionized water using Trichloroacetic Acid, 6.1 N Solution, approximately 100% (w/v), Sigma Stock No. 490-10.)
- M. 4 M Ammonium Hydroxide Solution (NH₄OH)
(Prepare 50 ml in deionized water using Ammonium Hydroxide, Sigma Prod. No. A-6899.)

PROCEDURE:

Prepare 0.5 ml Dowex 50W columns for each Test, Standard, and Blank.¹

Prepare a reaction cocktail by pipetting (in milliliters) the following reagents into a suitable container:

Deionized Water	3.50
Reagent A (Buffer)	2.00
Reagent B (Tyramine)	0.50
Reagent C (Asc)	0.50
Reagent D (Fum)	0.50
Reagent E (NEM)	1.50
Reagent I (Cat)	0.50

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PROCEDURE: (continued)

Mix by swirling and adjust to pH 5.0 at 37°C with either 1 M HCl or 1 M NaOH, if necessary.

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

	<u>Test</u>	<u>Std 1</u>	<u>Std 2</u>	<u>Blank</u>
Reaction Cocktail	0.90	0.90	0.90	0.90

Equilibrate to 37°C with agitation (using a metabolic shaker) while exposing to air in order to aerate the solution. Then add:

Deionized Water	-----	-----	0.05	0.10
Reagent J (Enzyme Soln.)	0.10	-----	-----	-----
Reagent H (Octo)	-----	0.10	0.05	-----

Mix by swirling and incubate with agitation for exactly 5 minutes. Then add:

Reagent L (TCA)	0.20	0.20	0.20	0.20
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Mix by inversion. Pour the contents of the Test, Standards, and Blank into Dowex 50W columns appropriately labeled. Wash each column 10 times using 0.5 ml of water for each washing and discard the washings.

Elute each column with 2.5 ml of Reagent M using 0.5 ml volumes and save the eluates for Test, Standards, and Blanks.

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

	<u>Test</u>	<u>Std 1</u>	<u>Std 2</u>	<u>Blank</u>
Test Eluate	2.50	-----	-----	-----
Standard 1 Eluate	-----	2.50	-----	-----
Standard 2 Eluate	-----	-----	2.50	-----
Blank Eluate	-----	-----	-----	2.50
Reagent F (Per)	0.25	0.25	0.25	0.25

Mix by swirling and incubate at 25°C for exactly 10 minutes. Then add:

Reagent G (Bisul)	0.25	0.25	0.25	0.25
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PROCEDURE: (continued)

Mix by swirling. Transfer to suitable cuvettes and record the absorbance at 330 nm for the Test, Standards, and Blank, using a suitable spectrophotometer.

CALCULATIONS:

Standard Determination:

$$r A_{330\text{nm}} = A_{330\text{nm}} \text{ Standard} - A_{330\text{nm}} \text{ Blank}$$

Sample Determination:

$$\text{Units/ml enzyme} = \frac{(A_{330\text{nm}} \text{ Test} - A_{330\text{nm}} \text{ Blank})(df)}{(5)(r A_{330\text{nm}}/\mu\text{moles/Std}) (0.1)}$$

df = Dilution factor

5 = Time (in minutes) of assay as per the Unit Definition

0.1 = Volume (in milliliters) of enzyme used

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

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

UNIT DEFINITION:

One unit will convert 1.0 μ mole of tyramine to octopamine per minute at pH 5.0 at 37°C.

FINAL ASSAY CONCENTRATIONS:

In a 1.00 ml reaction mix, the final concentrations are 200 mM sodium acetate, 20 mM tyramine, 10 mM sodium ascorbate, 10 mM sodium fumarate, 30 mM N-ethylmaleimide, 1750 units catalase and 0.015 - 0.030 unit dopamine β -hydroxylase.

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REFERENCES:

Nagatsu, T. and Udenfriend, S. (1972) *Clinical Chemistry*
18, 980-983.

NOTES:

1. A simple column can be constructed by putting a porous polyethylene plug in a 1 ml graduated disposable syringe. The Dowex 50W resin is made into a slurry using deionized water and poured into the column until the resin slurry settles to the 0.5 ml mark of the syringe.
2. This assay is based on the cited reference.
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.