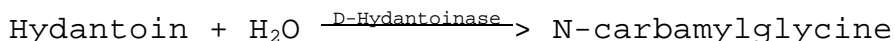


**Enzymatic assay of D-HYDANTOINASE
(EC 3.5.22)**

PRINCIPLE:



CONDITIONS: T = 40°C, pH = 9.0, A_{450nm}, Light path = 1 cm

METHOD: Spectrophotometric Stopped Rate Determination

REAGENTS:

- A. 100 mM Sodium Borate Buffer with 50 mM Sodium Chloride, pH 9.0 at 40°C
(Prepare 100 ml in deionized water using Boric Acid, Sigma Prod. No. B-0252 and Sodium Chloride, Sigma Prod. No. S-9625. Adjust to pH 9.0 at 40°C with 5 M NaOH.)
- B. 100 mM Hydantoin Solution (Hydantoin)
(Prepare 5 ml in Reagent A using Hydantoin, Sigma Prod. No. H-4879.)
- C. 12% (w/v) Trichloroacetic Acid Solution (TCA)
(Prepare 25 ml in deionized water using Trichloroacetic Acid, 6.1 N Solution, approx. 100% (w/v), Sigma Stock No. 490-10.)
- D. 10% (w/v) p-Dimethylaminobenzaldehyde Solution (DMABA)
(Prepare 5 ml in 6 M HCl using p-Dimethylaminobenzaldehyde, Sigma Prod. No. D-8904.)
- E. 250 mM Hydantoic Acid Solution (Hydant. Std)
(Prepare 5 ml in deionized water using Hydantoic Acid, Sigma Prod. No. H-3379.)
- F. Hydantoinase Enzyme Solution
(Immediately before use, prepare a solution containing 10 mg/ml of Hydantoinase in cold deionized water.)

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PROCEDURE:

Equilibrate Reagent B (Hydantoin) to 40°C in a water bath.

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

	<u>Test</u>	<u>Blank</u>
Reagent F (Enzyme Solution)	0.10	0.10

Equilibrate to 40°C. Then add:

Reagent B (Hydantoin) ¹	0.30	-----
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Cap the tubes and incubate at 40°C for exactly 10 minutes.

Then add:

Reagent C (TCA)	0.70	0.70
Reagent B (Hydantoin)	-----	0.30

Then add:

Reagent D (DMBA)	0.15	0.15
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Cap the tubes, mix by inversion and then centrifuge for 2 minutes in a microcentrifuge. Transfer the supernatant to suitable cuvettes and record the $A_{450\text{nm}}$ for the Test and Blank using a suitable spectrophotometer.

Standard Curve:

A standard curve is prepared by pipetting (in milliliters) the following reagents into suitable tubes:

	<u>Std 1</u>	<u>Std 2</u>	<u>Std 3</u>	<u>Std 4</u>	<u>Std Blank</u>
Reagent E (Hydant. Std)	0.02	0.04	0.08	0.10	0.00
Deionized Water	0.38	0.36	0.32	0.30	0.40
Reagent C (TCA)	0.70	0.70	0.70	0.70	0.70
Reagent D (DMABA)	0.15	0.15	0.15	0.15	0.15

Cap the tubes, mix by inversion and then centrifuge for 2 minutes in a microcentrifuge. Transfer the supernatant to suitable cuvettes and record the $A_{450\text{nm}}$ for the Standards and Standard Blank using a suitable spectrophotometer.

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CALCULATIONS:

Standard Curve:

$$r A_{450\text{nm}} \text{ Standard} = A_{450\text{nm}} \text{ Standard} - A_{450\text{nm}} \text{ Standard Blank}$$

Plot the $r A_{450\text{nm}}$ of the standards versus μmoles of Hydantoic acid.

Sample Determination:

$$r A_{450\text{nm}} \text{ Test} = A_{450\text{nm}} \text{ Test} - A_{450\text{nm}} \text{ Test Blank}$$

Determine the μmoles of hydantoic acid produced using the Standard Curve.

$$\text{Units/ml enzyme} = \frac{(\mu\text{moles of hydantoic acid produced})}{(10)(0.1)}$$

10 = Time (in minutes) of assay

0.1 = Volume (in milliliters) of enzyme used

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

$$\text{Units/g solid} = 1000 \times \text{units/mg solid}$$

UNIT DEFINITION:

One unit will catalyze the formation of 1 μmole of N-carbamylglycine from hydantoin per minute at pH 9.0 at 40°C.

FINAL ASSAY CONCENTRATION:

In a 0.4 ml reaction mix, the final concentrations are 75 mM boric acid, 38 mM sodium chloride, 75 mM hydantion, and 1 mg hydantoinase.

REFERENCE:

Morin, A. (1993), *Enzyme Microbial Technology* **15**, 208-214.

NOTES:

1. Reagent B (Hydantoin) must be equilibrated to 40°C immediately prior to addition.

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NOTES: (continued)

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.