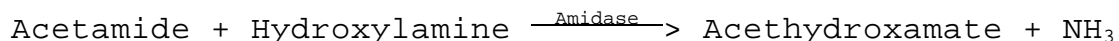


**Enzymatic Assay of AMIDASE
(EC 3.5.1.4)**

PRINCIPLE:



CONDITIONS: T = 37°C, pH = 7.2, A_{500nm}, Light path = 1 cm

METHOD: Colorimetric

REAGENTS:

- A. 100 mM Sodium Phosphate Buffer, pH 7.2 at 37°C
(Prepare 100 ml in deionized water using Sodium Phosphate, Monobasic, Anhydrous, Sigma Prod. No. S-0751. Adjust to pH 7.2 at 37°C with 1 M NaOH.)
- B. 400 mM Acetamide Solution (Acet)
(Prepare 10 ml in deionized water using Acetamide, Sigma Prod. No. A-0500. The solution is stable on ice for 2 - 3 hours. **PREPARE FRESH.**)
- C. 2 M Hydroxylamine Solution (Hydrox)
(Prepare 10 ml in deionized water using Hydroxylamine Hydrochloride, Sigma Prod. No. H-9876. Adjust to pH 7.2 at 37°C with 10 M NaOH.)
- D. Hydrochloric Acid (HCl)
(Use Hydrochloric Acid, Sigma Prod. No. H-7020.)
- E. 60% (w/v) Ferric Chloride Solution (FeCl₃)
(Prepare 15 ml in deionized water using Ferric Chloride, Hexahydrate, Sigma Prod. No. F-2877.)
- F. Color Reagent Solution (CRS)
(Prepare 100 ml by adding 5.7 ml of Reagent D to 10 ml of Reagent E. Dilute to 100 ml with deionized water.)
- G. 7 mM Dithiothreitol Solution (Enz Dil)
(Prepare 10 ml in Reagent A using DL-Dithiothreitol, Sigma Prod. No. D-0632.)

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REAGENTS: (continued)

- H. 100 mM Acetamide Solution
(Prepare 5 ml in deionized water using Acetamide, Sigma Prod. No. A-0500. **PREPARE FRESH.**)
- I. 5 M Hydroxylamine Solution
(Prepare 10 ml in 4.5 M NaOH using Hydroxylamine Hydrochloride, Sigma Prod. No. H-9876.)
- J. 5 mM Acethydroxamate Standard Solution (Std Soln)
(Prepare 100 ml by adding 5 ml of Reagent H to 10 ml of Reagent I. Heat in a boiling water bath for 15 minutes. Cool the solution and adjust to pH 7.2 at 37°C with 5 M HCl, if necessary. Dilute up to 100 ml with deionized water.)
- K. Amidase Enzyme Solution
(Immediately before use, prepare a solution containing 3 - 6 units/ml of Amidase in cold Reagent G.)

PROCEDURE:

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

Reagent A (Buffer)	16.00
Reagent B (Acet)	8.00
Reagent C (Hydrox)	8.00

Mix by swirling and store on ice.

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

	Test							Std
	<u>Test</u>	<u>Blank</u>	<u>Std 1</u>	<u>Std 2</u>	<u>Std 3</u>	<u>Std 4</u>	<u>Std 5</u>	<u>Blank</u>
Reaction Cocktail	1.90	1.90	----	----	----	----	----	----
Reagent J (Std Soln)	----	----	0.20	0.50	1.00	1.50	2.00	----
Deionized Water	----	----	----	----	1.80	----	1.50	1.00
								0.50

								2.00

Equilibrate to 37°C. Then add:

Reagent K (Enz Soln)	0.10	----	----	----	----	----	----	----
Reagent G (Enz Dil)	----	0.10	----	----	----	----	----	----

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PROCEDURE: (continued)

Immediately mix by swirling and incubate at 37°C for exactly 5 minutes. Then add:

	<u>Test</u>	<u>Test Blank</u>	<u>Std 1</u>	<u>Std 2</u>	<u>Std 3</u>	<u>Std 4</u>	<u>Std 5</u>	<u>Std Blank</u>
Reagent F (CRS)	4.00	4.00	4.00	4.00	4.00	4.00	4.00	4.00

Mix by swirling and transfer the solutions to suitable cuvettes. Tap the cuvettes to dislodge any gas bubbles from the container walls. Record the A_{500nm} for the Test, Test Blank, Standards and Standard Blank using a suitable spectrophotometer.

CALCULATIONS:

Standard Curve:

$$r A_{500nm} \text{ Standard} = A_{500nm} \text{ Std} - A_{500nm} \text{ Std Blank}$$

Plot the $r A_{500nm}$ of the Standard vs μmoles of Acethydroxamate.

Sample Determination:

$$r A_{500nm} \text{ Sample} = A_{500nm} \text{ Test} - A_{500nm} \text{ Test Blank}$$

Determine the μmoles of Acethydroxamate produced using the Standard Curve.

$$\text{Units/ml enzyme} = \frac{(\mu\text{moles of acethydroxamate produced}) (df)}{(5) (0.1)}$$

df = Dilution factor

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

0.1 = Volume (in milliliter) of enzyme used

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

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

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UNIT DEFINITION:

One unit will convert 1.0 μ mole of acetamide and hydroxylamine to acethydroxamate and ammonia per minute at pH 7.2 at 37°C.

FINAL CONCENTRATION:

In a 2.00 ml reaction mix, the final concentrations are 53 mM sodium phosphate, 95 mM acetamide, 475 mM hydroxylamine and 0.3 - 0.6 unit amidase.

NOTES:

1. 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.