

Enzymatic Assay of α -MANNOSIDASE (EC 3.2.1.24)

PRINCIPLE:

p-Nitrophenyl- α -D-Mannoside + H₂O $\xrightarrow{\alpha\text{-Mannosidase}}$ D-Mannose + p-Nitrophenol

CONDITIONS: T = 25°C, pH = 4.5, A_{405nm}, Light path = 1 cm

METHOD: Spectrophotometric Stop Rate Determination

REAGENTS:

- A. 100 mM Citrate Buffer, pH 4.5 at 25°C
(Prepare 100 ml in deionized water using Citric Acid, Free Acid, Monohydrate, Prod. No. C-7129. Adjust to pH 4.5 at 25°C with 1 M NaOH.)
- B. 10 mM p-Nitrophenyl α -D-Mannoside Solution (PNP-Man)
(Prepare 5 ml in deionized water using p-Nitrophenyl α -D-Mannopyranoside, Prod. No. N-2127.)
- C. 200 mM Borate Buffer, pH 9.8 at 25°C
(Prepare 100 ml in deionized water using Boric Acid, Prod. No. B-0252. Adjust to pH 9.8 at 25°C with 1 M NaOH.)
- D. α -Mannosidase Enzyme Solution
(Immediately before use, prepare a solution containing 0.05 - 0.1 unit/ml of α -Mannosidase in cold deionized water.)

PROCEDURE:

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

	<u>Test</u>	<u>Blank</u>
Deionized Water	-----	0.10
Reagent A (Buffer)	0.50	0.50
Reagent B (PNP-Man)	0.50	0.50

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PROCEDURE: (continued)

Mix by inversion and equilibrate to 25°C. Then add:

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

Immediately mix by inversion and incubate at 25°C for exactly 5 minutes in a suitable spectrophotometer. Then add:

Reagent C (Borate)	2.00	2.00
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Mix by inversion and record the $A_{405\text{nm}}$ for both the Test and Blank.

CALCULATIONS:

$$\text{Units/mg enzyme} = \frac{(\Delta A_{405\text{nm}}/\text{min Test} - \Delta A_{405\text{nm}}/\text{min Blank}) (3.1)}{(5) (18.5) (\text{mg enzyme/RM})}$$

3.1 = Total volume (in milliliters) of Solution

5 = Time of assay (Unit definition)

18.5 = Millimolar extinction coefficient of p-Nitrophenol
at 405 nm

RM = Reaction Mix

UNIT DEFINITION:

One unit will hydrolyze 1.0 μmole of p-nitrophenyl α -D-mannoside to p-nitrophenol and D-mannose per minute at pH 4.5 at 25°C.

FINAL ASSAY CONCENTRATION:

In a 1.10 ml reaction mix, the final concentrations are 45 mM citric acid, 4.5 mM p-nitrophenyl α -D-mannoside and 0.005 - 0.01 unit α -mannosidase.

REFERENCES:

Li, Y.-T. (1967) *J. Biol. Chem.* **242**, 5474-5480.

Lee, Y.C. (1972) *Methods in Enzymology* **XXVIII**, 699-702.

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NOTES:

1. All product and stock numbers, unless otherwise indicated, are Sigma product and stock numbers.

This procedure is for informational purposes. For a current copy of Sigma's quality control procedure contact our Technical Service Department.