Enzymatic Assay of ß-MANNOSIDASE
(EC 3.2.1.25)

PRINCIPLE:

p-Nitrophenyl ß-D-Mannoside + H_2O $\xrightarrow{\text{ß-Mannosidase}}$ D-Mannose + p-Nitrophenol

CONDITIONS: T = 25°C, pH = 4.0, A_{400nm}, Light path = 1 cm

METHOD: Spectrophotometric Stop Rate Determination

REAGENTS:

A. 100 mM Citrate Buffer, pH 4.0 at 25°C
(Prepare 100 ml in deionized water using Citric Acid, Free Acid, Monohydrate, Prod. No. C-7129. Adjust to pH 4.0 at 25°C with 1 M NaOH.)

B. 10 mM p-Nitrophenyl ß-D-Mannoside Solution (PNP-ß-Man)
(Prepare 5 ml in deionized water using p-Nitrophenyl ß-D-Mannopyranoside, Prod. No. N-1268.)

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. ß-Mannosidase Enzyme Solution
(Immediately before use, prepare a solution containing 0.05 - 0.1 unit/ml of ß-Mannosidase in cold deionized water.)

PROCEDURE:

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

	Test	Blank
Reagent A (Buffer)	0.40	0.40
Reagent D (Enzyme Solution)	0.10	------

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

PROCEDURE: (continued)

	Test	Blank
Reagent B (PNP-ß-Man)	0.50	0.50

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

Reagent C (Borate)	3.00	3.00
Reagent D (Enzyme Solution)	------	0.10

Mix by swirling and transfer to suitable cuvettes. Record the A_{400nm} for both the Test and Blank using a suitable spectrophotometer.

CALCULATIONS:

$$\text{Units/mg enzyme} = \frac{(A_{400nm}/\text{min Test} - A_{400nm}/\text{min Blank})\ (4)}{(10)\ (18)\ (\text{mg enzyme/RM})}$$

4 = Total volume (in milliliters) of Solution
10 = Time of assay (in minutes) as per the Unit Definition
18 = Millimolar extinction coefficient of p-Nitrophenol at 400 nm
RM = Reaction Mix

UNIT DEFINITION:

One unit will hydrolyze 1.0 µmole of p-nitrophenyl ß-D-mannopyranoside to p-nitrophenol and D-mannopyranoside per minute at pH 4.0 at 25°C.

FINAL ASSAY CONCENTRATION:

In a 1.00 ml reaction mix, the final concentrations are 40 mM citric acid, 5.0 mM p-nitrophenyl ß-D-mannoside and 0.005 - 0.01 unit ß-mannosidase.

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REFERENCES:

Sugahara, K., and Yamashina, I. (1972) *Methods in Enzymology* **XXVIII**, Part B, 769-772.

Tarentino, A.L., and Maley, F. (1972) *Methods in Enzymology* **XXVIII**, Part B, 772-776.

Sukeno, T., Tarentino, A.L., Plummer, Jr., T.H., and Maley, F. (1972) *Methods in Enzymology* **XXVIII**, Part B, 777-782.

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.