

**Enzymatic Assay of  $\beta$ -MANNOSIDASE  
(EC 3.2.1.25)**

**PRINCIPLE:**

p-Nitrophenyl  $\beta$ -D-Mannoside + H<sub>2</sub>O  $\xrightarrow{\beta\text{-Mannosidase}}$  D-Mannose + p-Nitrophenol

**CONDITIONS:** T = 25°C, pH = 4.0, A<sub>400nm</sub>, 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  $\beta$ -D-Mannoside Solution (PNP- $\beta$ -Man)  
(Prepare 5 ml in deionized water using p-Nitrophenyl  $\beta$ -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.  $\beta$ -Mannosidase Enzyme Solution  
(Immediately before use, prepare a solution containing 0.05 - 0.1 unit/ml of  $\beta$ -Mannosidase in cold deionized water.)

**PROCEDURE:**

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

	<u>Test</u>	<u>Blank</u>
Reagent A (Buffer)	0.40	0.40
Reagent D (Enzyme Solution)	0.10	-----

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

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**PROCEDURE:** (continued)

	<u>Test</u>	<u>Blank</u>
Reagent B (PNP- $\beta$ -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  $A_{400\text{nm}}$  for both the Test and Blank using a suitable spectrophotometer.

**CALCULATIONS:**

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

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  $\mu\text{mole}$  of p-nitrophenyl  $\beta$ -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  $\beta$ -D-mannoside and 0.005 - 0.01 unit  $\beta$ -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.**