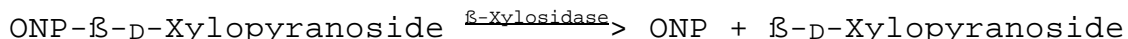


**Enzymatic Assay of  $\beta$ -XYLOSIDASE  
(EC 3.2.1.37)**

**PRINCIPLE:**



Abbreviations used:

ONP = o-Nitrophenol

**CONDITIONS:** T = 25°C, pH = 5.0,  $A_{410\text{nm}}$ , Light path = 1 cm

**METHOD:** Spectrophotometric Stop Rate Determination

**REAGENTS:**

- A. 100 mM Citrate Buffer, pH 5.0 at 25°C  
(Prepare 100 ml in deionized water using Citric Acid, Free Acid, Prod. No. C-0759. Adjust to pH 5.0 at 25°C with 1 M NaOH.)
- B. 10 mM o-Nitrophenyl  $\beta$ -D-Xylopyranoside Solution  
(ONP- $\beta$ -D-Xylopyranoside)  
(Prepare 10 ml in deionized water using o-Nitrophenyl  $\beta$ -D-Xylopyranoside, Prod. No. N-3629.)
- 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.  $\beta$ -Xylosidase Enzyme Solution  
(Prepare a solution containing 1-2 units/ml of  $\beta$ -Xylosidase in cold deionized water.)

**PROCEDURE:**

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

	<u>Test</u>	<u>Blank</u>
Reagent A (Citrate Buffer)	0.45	0.45
Reagent B (ONP- $\beta$ -D-Xylopyranoside)	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)	0.05	-----
Deionized Water	-----	0.05

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

Reagent C (Borate Buffer)	3.00	3.00
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Mix by inversion and record the  $A_{410\text{nm}}$  for both the Test and Blank, using a suitably thermostatted spectrophotometer.

**CALCULATIONS:**

$$\text{Units/mg enzyme} = \frac{(\Delta A_{410\text{nm}} \text{ Test} - \Delta A_{410\text{nm}} \text{ Blank}) (4.0)}{(10) (4.6) (\text{mg enzyme/RM})}$$

4.0 = Total volume (in milliliters) of solution

10 = Time of assay in minutes (unit definition)

4.6 = Millimolar extinction coefficient of o-nitrophenol  
at 410 nm and pH 9.8

RM = Reaction Mix

**UNIT DEFINITION:**

One unit will hydrolyze 1.0  $\mu\text{mole}$  of o-nitrophenyl  $\beta$ -D-xyloside to o-nitrophenol and D-xylose per minute at pH 5.0 at 25°C.

**FINAL ASSAY CONCENTRATION:**

In a 1.00 ml reaction mix, the final concentrations are 45 mM citric acid, 5.0 mM ONP- $\beta$ -D-xylopyranoside, and 0.05 - 0.10 unit  $\beta$ -xylosidase.

**NOTES:**

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

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