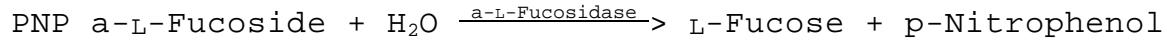


**Enzymatic Assay of  $\alpha$ -L-FUCOSIDASE  
(EC 3.2.1.51)**

**PRINCIPLE:**



Abbreviations used:

PNP  $\alpha$ -L-Fucoside = p-Nitrophenyl  $\alpha$ -L-Fucoside

**CONDITIONS:**  $T = 25^\circ C$ ,  $pH = 5.5$ ,  $A_{400nm}$ , Light path = 1 cm

**METHOD:** Spectrophotometric Stop Rate Determination

**REAGENTS:**

- A. 100 mM Sodium Citrate Buffer, pH 5.5 at 25°C  
(Prepare 100 ml in deionized water using Citric Acid, Trisodium Salt, Prod. No. C-7254. Adjust to pH 5.5 at 25°C with 1 M HCl. **PREPARE FRESH.**)
- B. 10 mM p-Nitrophenyl  $\alpha$ -L-Fucopyranoside Solution (PNP-FUC)  
(Prepare 5 ml in deionized water using p-Nitrophenyl  $\alpha$ -L-Fucopyranoside, Prod. No. N-3628.)
- C. 200 mM Borate Solution, pH 9.8 at 25°C (Borate)  
(Prepare 20 ml in deionized water using Boric Acid, Prod. No. B-0252. Adjust to pH 9.8 at 25°C with 1 M NaOH.)
- D.  $\alpha$ -L-Fucosidase Enzyme Solution  
(Immediately before use, prepare a solution containing 0.02 - 0.04 unit/ml of  $\alpha$ -L-Fucosidase in cold Reagent A.)

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**PROCEDURE:**

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

	<u>Test</u>	<u>Blank</u>
Reagent A (Buffer)	0.40	0.40
Reagent B (PNP-FUC)	0.50	0.50

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

Reagent D (Enzyme Solution)	0.10	-----
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Immediately mix by inversion and incubate for exactly 10 minutes at 25°C. Then add:

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

Mix by inversion and transfer to suitable cuvettes. Record the  $A_{400\text{nm}}$  for both the Test and Blank in a suitable spectrophotometer.

**CALCULATIONS:**

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

18 = Millimolar extinction coefficient of p-Nitrophenol at 400 nm

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

4 = Total volume of enzyme assay

RM = Reaction mix

**UNIT DEFINITION:**

One unit will hydrolyze 1.0  $\mu$ mole of p-nitrophenyl  $\alpha$ -L-fucoside to p-nitrophenol and L-fucose per minute at pH 5.5 at 25°C.

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**FINAL ASSAY CONCENTRATIONS:**

In a 1.00 ml reaction mix, the final concentrations are 50 mM citrate, 5.0 mM p-nitrophenyl  $\alpha$ -L-fucoside and 0.002 - 0.004 unit  $\alpha$ -L-fucosidase.

**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.**