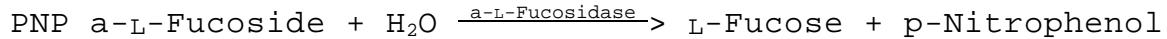


**Enzymatic Assay of α -L-FUCOSIDASE
(EC 3.2.1.51)**

PRINCIPLE:



Abbreviations used:

PNP α -L-Fucoside = p-Nitrophenyl α -L-Fucoside

CONDITIONS: T = 37°C, pH = 4.5, A_{400nm}, Light path = 1 cm

METHOD: Spectrophotometric Stop Rate Determination

REAGENTS:

- A. 50 mM Sodium Citrate Buffer, pH 4.5 at 37°C
(Prepare 100 ml in deionized water using Citric Acid, Trisodium Salt, Prod. No. C-7254. Adjust to pH 4.5 at 37°C with HCl. **PREPARE FRESH.**)
- B. 2.0 mM p-Nitrophenyl α -L-Fucopyranoside Solution (PNP-FUC)
(Prepare 5 ml in Reagent A using p-Nitrophenyl α -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. α -L-Fucosidase Enzyme Solution
(Immediately before use, prepare a solution containing 0.50 - 1.0 unit/ml of α -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 B (PNP-FUC)	0.25	0.25

Mix by inversion and equilibrate to 37°C. Then add:

Reagent D (Enzyme Solution)	0.10	-----
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Immediately mix by inversion and incubate for exactly 15 minutes at 37°C. Then add:

Reagent C (Borate)	2.20	2.20
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:

$$\text{Units/mg enzyme} = \frac{(A_{400\text{nm}} \text{ Test} - A_{400\text{nm}} \text{ Blank}) (2.55)}{(17.7) (15) (\text{mg enzyme/RM})}$$

17.7 = Millimolar extinction coefficient of p-Nitrophenol at 400 nm¹

2.55 = Total volume of enzyme assay

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

RM = Reaction mix

UNIT DEFINITION:

One unit will hydrolyze 1.0 μ mole of p-nitrophenyl α -L-fucoside to p-nitrophenol and L-fucose per minute at pH 4.5 at 37°C.

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FINAL ASSAY CONCENTRATIONS:

In a 0.35 ml reaction mix, the final concentrations are 50 mM citrate, 1.4 mM p-nitrophenyl α -L-fucoside and 0.05 - 0.10 unit α -L-fucosidase.

REFERENCES:

Yano, T., et al. (1985) *Agricultural Biological Chemistry* **49**, 3179.

Yamamoto, K., et al. (1986) *Agricultural Biological Chemistry*, **50**, 1689.

NOTES:

1. The millimolar extinction coefficient is cited in Yano, T. et al. (1985) *Agricultural Biological Chemistry* **49**, 3179.
2. 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.