

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

PRINCIPLE:

PNP α -L-Fucoside + H₂O $\xrightarrow{\alpha\text{-L-Fucosidase}}$ L-Fucose + p-Nitrophenol

Abbreviation used:

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

CONDITIONS: T = 25°C, pH = 6.5, A_{400nm}, Light path = 1 cm

METHOD: Spectrophotometric Stop Rate Determination

REAGENTS:

- A. 100 mM Sodium Citrate Buffer, pH 6.5 at 25°C
(Prepare 100 ml in deionized water using Citric Acid, Trisodium Salt, Dihydrate, Sigma Prod. No. C-7254. Adjust to pH 6.5 at 25°C with 1 M HCl. **PREPARE FRESH.**)
- B. 10 mM p-Nitrophenyl α -L-Fucopyranoside Solution (PNP-FUC)
(Prepare 5 ml in deionized water using p-Nitrophenyl α -L-Fucopyranoside, Sigma Prod. No. N-3628. Heat gently to dissolve.)
- C. 200 mM Borate Solution, pH 9.8 at 25°C (Borate)
(Prepare 50 ml in deionized water using Boric Acid, Sigma 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 0.05 - 0.10 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 A (Buffer)	0.40	0.40
Reagent B (PNP-FUC)	0.50	0.50

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

Reagent D (Enzyme Solution)	0.10	-----
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Immediately mix by swirling 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 swirling and transfer to suitable cuvettes. Record the $A_{400\text{nm}}$ for both the Test and Blank in a suitable spectrophotometer.

CALCULATIONS:

$$\text{Units/ml enzyme} = \frac{(A_{400\text{nm}} \text{ Test} - A_{400\text{nm}} \text{ Blank})(4)(\text{df})}{(18)(10)(0.1)}$$

4 = Total volume (in milliliters) of enzyme assay

df = Dilution factor

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

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

0.1 = Volume (in milliliter) of enzyme used

$$\text{Units/mg protein} = \frac{\text{units/ml enzyme}}{\text{mg protein/ml enzyme}}$$

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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 6.5 at 25°C.

FINAL ASSAY CONCENTRATIONS:

In a 1.00 ml reaction mix, the final concentrations are 50 mM citrate, 5.0 mM p-nitrophenyl α -L-fucoside and 0.005 - 0.010 unit α -L-fucosidase.

NOTES:

1. Where Sigma Product or Stock numbers are specified, equivalent reagents may be substituted.

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