Enzymatic Assay of a-L-FUCOSIDASE
(EC 3.2.1.51)

PRINCIPLE:

PNP a-L-Fucoside + H_2O $\xrightarrow{\text{a-L-Fucosidase}}$ L-Fucose + p-Nitrophenol

Abbreviations used:
PNP a-L-Fucoside = p-Nitrophenyl a-L-Fucoside

CONDITIONS: T = 25°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 a-L-Fucopyranoside Solution (PNP-FUC)
(Prepare 5 ml in deionized water using p-Nitrophenyl a-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. a-L-Fucosidase Enzyme Solution
(Immediately before use, prepare a solution containing 0.02 - 0.04 unit/ml of a-L-Fucosidase in cold Reagent A.)

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PROCEDURE:

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

	Test	Blank
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	------

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_{400nm} for both the Test and Blank in a suitable spectrophotometer.

CALCULATIONS:

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

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 µmole of p-nitrophenyl a-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 a-L-fucoside and 0.002 - 0.004 unit a-L-fucosidase.

NOTES:

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