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 = 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 a-L-Fucopyranoside Solution (PNP-FUC)
(Prepare 5 ml in Reagent A 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 0.50 - 1.0 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 B (PNP-FUC)	0.25	0.25

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

Reagent D (Enzyme Solution)	0.10	------

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

CALCULATIONS:

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

17.7 = Millimolar extinction coefficient of p-Nitrophenol at 400 nm[1]
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 µmole of p-nitrophenyl a-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 a-L-fucoside and 0.05 - 0.10 unit a-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.