Enzymatic Assay of L-FUCOSE DEHYDROGENASE
From Pseudomonas sp.

PRINCIPLE:

$$\text{L-Fucose} + \text{ß-NADP} \xrightarrow{\text{L-Fucose Dehydrogenase}} \text{L-Fucono-1,5-lactone} + \text{ß-NADPH}$$

Abbreviations:
ß-NADP = ß-Nicotinamide Adenine Dinucleotide Phosphate, Oxidized Form
ß-NADPH = ß-Nicotinamide Adenine Dinucleotide Phosphate, Reduced Form

CONDITIONS: T = 37°C, pH = 9.5, A_{340nm}, Light path = 1 cm

METHOD: Continuous Spectrophotometric Rate Determination

REAGENTS:

A. 120 mM Tris, 120 mM Imidazole and 100 mM Acetate HCl Buffer, pH 9.5 at 37°C
(Prepare 100 ml in deionized water using Trizma Base, Sigma Prod. No. T-1503, Imidazole, Sigma Prod. No. I-0250, and Sodium Acetate, Trihydrate, Sigma Prod. No. S-8625. Adjust to pH 9.5 at 37°C with 5 M HCl.)

B. 150 mM L-Fucose Solution (Fucose)
(Prepare 25 ml in Reagent A using L(-)Fucose, Sigma Prod. No. F-2252. **PREPARE FRESH.**)[1]

C. 15 mM ß-Nicotinamide Adenine Dinucleotide Phosphate Solution (ß-NADP)
(Prepare 5 ml in cold deionized water using ß-Nicotinamide Adenine Dinucleotide Phosphate, Sodium Salt, Sigma Prod. No. N-0505.)

D. L-Fucose Dehydrogenase Enzyme Solution
(Immediately before use, prepare a solution containing 0.15 - 0.25 unit/ml of L-Fucose Dehydrogenase in cold deionized water.)

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PROCEDURE:

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

	Test	Blank
Reagent A (Buffer)	2.50	2.50
Reagent B (Fucose)	0.20	------
Reagent C (ß-NADP)	0.20	0.20
Deionized Water	------	0.20

Mix by inversion and equilibrate to 37°C. Monitor the A_{340nm} until constant, using a suitably thermostatted spectrophotometer. Then add:

Reagent D (Enzyme Solution)	0.10	0.10

Immediately mix by inversion and record the increase in A_{340nm} for approximately 10 minutes. Obtain the ΔA_{340nm}/minute using the maximum linear rate for both the Test and Blank.

CALCULATIONS:

$$\text{Units/ml enzyme} = \frac{(\Delta A_{340nm}/\text{min Test} - \Delta A_{340nm}/\text{ min Blank})(3)(df)}{(6.22)(0.1)}$$

3 = Volume (in milliliters) of assay
df = Dilution factor
6.22 = Millimolar extinction coefficient of ß-NADPH at 340 nm 0.1 = Volume (in milliliter) of enzyme used

$$\text{Unit/mg solid} = \frac{\text{units/ml enzyme}}{\text{mg solid/ml enzyme}}$$

UNIT DEFINITION:

One unit will oxidize 1.0 µmole of L-fucose to L-fucono-1,5-lactone per minute at pH 9.5 at 37°C in the presence of NADP.

FINAL ASSAY CONCENTRATION:

In a 3.00 ml reaction mix, the final concentrations are 108 mM Tris, 108 mM imidazole, 90 mM sodium acetate, 10 mM L-fucose, 1.0 mM ß-nicotinamide adenine dinucleotide phosphate and 0.015 - 0.025 unit L-fucose dehydrogenase.

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REFERENCE:

Horiuchi, T., Suzuki, T., Hiruma, M. and Saito, N. (1989) *Agricultural and Biological Chemistry*, 53, 1493-1501

NOTES:

1. This solution should be used within 1 hour. Store on ice.

2. This assay is based on the cited reference.

3. 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.