Enzymatic Assay of L-FUCOSE DEHYDROGENASE
(EC 1.1.1.122)
from Porcine Liver

PRINCIPLE:

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

Abbreviations:
ß-NAD = ß-Nicotinamide Adenine Nucleotide, Oxidized Form
ß-NADH = ß-Nicotinamide Adenine Nucleotide, Reduced Form

CONDITIONS: T = 37°C, pH = 8.7, A_{340nm}, Light path = 1 cm

METHOD: Continuous Spectrophotometric Rate Determination

REAGENTS:

A. 55 mM Tris HCl Buffer, pH 8.7 at 37°C
(Prepare 50 ml in deionized water using Trizma Base, Sigma Prod. No. T-1503. Adjust to pH 8.7 at 37°C with 1 M HCl.)

B. 5.5 mM a-L-Fucose Solution (Fucose)
(Prepare 25 ml in Reagent A using a-L(-)Fucose, Sigma Prod. No. F-2252. **PREPARE FRESH**.)

C. 30 mM ß-Nicotinamide Adenine Dinucleotide Solution (ß-NAD)
(Prepare 1 ml in deionized water using ß-Nicotinamide Adenine Dinucleotide, Sigma Prod. No. N-7004. **PREPARE FRESH**.)

D. L-Fucose Dehydrogenase Enzyme Solution
(Immediately before use, prepare a solution containing 0.2 - 0.5 unit/ml of L-Fucose Dehydrogenase in cold Reagent A.)

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PROCEDURE:

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

	Test	Blank
Reagent B (Fucose)	2.80	2.80
Reagent C (ß-NAD)	0.10	0.10

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

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

Immediately mix by inversion and record the increase in A_{340nm} for approximately 5 minutes. Obtain the $r\ A_{340nm}$/minute using the maximum linear rate for both the Test and Blank.

CALCULATIONS:

$$\text{Units/mg enzyme} = \frac{r\ A_{340nm}/\text{min Test} - r\ A_{340nm}/\text{min Blank}}{(6.22)\ (\text{mg enzyme/ml RM})}$$

6.22 = Millimolar extinction coefficient of ß-NADH at 340 nm
RM = Reaction Mix

UNIT DEFINITION:

One unit will oxidize 1.0 µmole of L-fucose to L-fucono-1,5-lactone per minute at pH 8.7 at 37°C.

FINAL ASSAY CONCENTRATION:

In a 3.00 ml reaction mix, the final concentrations are 53 mM Tris, 5.1 mM L-fucose, 1.0 mM ß-NAD and 0.02 -.05 unit L-fucose dehydrogenase.

REFERENCE:

Schachter, H. et al., (1969) *Journal of Biological Chemistry, 244*, 4785-4792.

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NOTES:

1. This assay is a modification of the enzyme assay described in the cited reference.

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.