Enzymatic Assay of L-Fucose Dehydrogenase
(EC 1.1.1.122)
from Porcine Liver

PRINCIPLE:

L-Fucose + β-NAD > L-Fucono-1,5-lactone + β-NADH

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

CONDITIONS:  T = 37°C, pH = 8.7, A\textsubscript{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. 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. PREPARE FRESH.)

C.  30 mM β-Nicotinamide Adenine Dinucleotide Solution (β-NAD)
(Prepare 1 ml in deionized water using β-Nicotinamide Adenine Dinucleotide. 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:

<table>
<thead>
<tr>
<th>Reagent B (Fucose)</th>
<th>Test</th>
<th>Blank</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>2.80</td>
<td>2.80</td>
</tr>
<tr>
<td>Reagent C (ß-NAD)</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>0.10</td>
<td>0.10</td>
</tr>
</tbody>
</table>

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

<table>
<thead>
<tr>
<th>Reagent A (Buffer)</th>
<th>Test</th>
<th>Blank</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>------</td>
<td>0.10</td>
</tr>
<tr>
<td>Reagent D (Enzyme Solution)</td>
<td>0.10</td>
<td>------</td>
</tr>
</tbody>
</table>

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 - 0.05 unit L-fucose dehydrogenase.

REFERENCE:

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NOTES:

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

This procedure is for informational purposes.