Protocol

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Enzymatic Assay of ι-Fucose Dehydrogenase

Introduction

The monosaccharide ι-fucose (6-deoxy-L-galactose) is a component of many N- and O-linked glycans and glycolipids and is produced by microorganisms, plants, and animals. It is a common terminal modification of glycan structures and has a role in many different processes. In addition, a change in ι-fucose metabolism has been linked to different diseases including breast cancer, ovarian cancer, colorectal adenocarcinoma, leukemia, brain tumors, cirrhosis, meningitis, tuberculosis, and cardiovascular disorders. One way to study ι-Fucose is to measure its metabolism by ι-fucose dehydrogenase, the main enzyme responsible for ι -fucose catabolism. Here, we outline a procedure in which the oxidation of ι-fucose by ι-fucose dehydrogenase is measured by UV spectroscopy and a reaction rate is established. This can be used in other applications to determine rates of reaction for other enzymes that utilize ι-fucose.

Materials

- Tris (GoldBio Catalog # T-400)
- Imidazole (GoldBio Catalog # I-902)
- Acetate HCl buffer
- 5M HCl
- L-Fucose (GoldBio Catalog # F-260)
- Molecular biology grade water
- Ice
- β-NADP
- Spectrophotometer with a path length of 1 cm

Preparation of Buffers and Solutions:

- Prepare these reagents with the final concentrations listed. The buffer should have a pH of 9.5 at a temperature of 37°C. Adjust the pH with 5M HCl if necessary.
    a. 120mM Tris
    b. 120mM Imidazole
    c. 100mM Acetate HCl Buffer

For the ι -Fucose Solution:

- Prepare a fresh solution with a final concentration of 150mM in molecular biology grade water.

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- Solution should be used within an hour of preparation and should be kept on ice at all times.

For the β-NADP Solution:

- Prepare a solution of β-Nicotinamide Adenine Dinucleotide Phosphate (β-NADP) with a final concentration of 15mM in cold molecular biology grade water.

For the ι -Fucose Dehydrogenase Enzyme Solution:

- Prepare a solution of isolated ι -Fucose Dehydrogenase in cold molecular biology grade water immediately before use.

Method

1. Into a cuvette prepare the following test and blank samples (see Table 1). Invert to mix and allow to equilibrate to 37°C. Monitor the $A_{340\ nm}$ until constant.

Table 1. Preparation of test and blank samples.

Reagent	Test (mL)	Blank (mL)
Buffer	2.50	2.50
ι -Fucose	0.20	N/A
β-NADP	0.20	0.20
Molecular Biology Grade Water	N/A	0.20

2. Add 0.10 mL of the enzyme solution to the test and blank, quickly invert to mix and measure the change in $A_{340\ nm}$/min for 15 minutes.

Note: In the presence of NADP, 1 unit of ι -Fucose Dehydrogenase will typically oxidize 1.0µM of ι -Fucose into ι -Fucono-1,5-lactone per minute (at a pH of 9.5 and 37°C).

Calculations

$$\frac{Units}{mL}enzyme = \frac{(\Delta A340nm/minTest - \Delta A340nm/minBlank)(3)(df)}{(6.22)(0.1)}$$

3 = Volume (in ml) of assay
Df = dilution factor (if necessary)
6.22 = Millimolar extinction coefficient of β-NADPH at 340 nm
0.1 = Volume (in ml) of enzyme solution used

Associated Products

- Imidazole (GoldBio Catalog # I-902)
- L-Fucose (GoldBio Catalog # F-260)
- Tris (GoldBio Catalog # T-400)

References

Becker, D. J. and Lowe, J. B. (2003). Fucose: Biosynthesis and biological function in mammals. *Glycobiology,* 13(7). Doi:10.1093/glycob/cwg054.

Horiuchi, T., Sizuki, T., Hiruma, M. and Saito, N. (1989) Purification and Characterization of ι –Fucose (ι –Galactose) Dehydrogenase from *Pseudomonas sp.* No. 1143. *Agricultural and Biological Chemistry*, 53, 1493-1501. Doi: 10.1080/00021369.1989.10869508.