Invertase Assay Kit

Introduction

Invertase (β-fructofuranosidase) is an enzyme that catalyzes the hydrolysis of sucrose to fructose and glucose. Invertase is produced by certain plants, microorganisms, and honey bees. In this kit, invertase activity is determined by a coupled enzyme assay in which invertase cleaves sucrose to glucose and fructose, resulting in a colorimetric (570 nm)/fluorometric (**λ**ex = 535/**λ**em = 587 nm) product, proportional to the invertase activity present. This kit is suitable for the detection of invertase activity in biological and environmental samples. One unit of Invertase is the amount of enzyme that will catalyze the formation of 1.0 µmole of glucose per minute at pH 4.5 under the assay conditions.

Materials

- The kit is sufficient for 100 assays in 96 well plates.
- 10x Reaction Buffer, pH 4.5 12 mL
- Assay Buffer 10 mL
- Glucose Standard 1 mL
- 10x Sucrose 1.5 mL
- Enzyme Mix 120 µL
- Dye Reagent 120 µL
- 96 well flat-bottom plate
- Fluorescence or spectrophotometric multiwell plate reader
- 10 kDa Molecular Weight Cut-Off (MWCO) Spin Filter

Procedure

1. Prep

1. Bring all reagents to room temperature prior to use.
2. Briefly centrifuge vials before opening.
3. Use ultrapure water for the preparation of reagents.
4. To maintain reagent integrity, avoid repeated freeze/thaw cycles.
5. Prior to assay, dilute the 10x Reaction Buffer and the 10x Sucrose solution to 1x by mixing one volume of each 10x solution with 9 volumes of water.
6. The 1x solutions should be used in the assays.

7. This assay can be performed at either 30 degC or 37 degC

Assay Reaction

8. Transfer 40 µL of the appropriate standards and 40 µL of samples into separate wells of a 96 well plate.

9. Transfer 40 µL of diluted 1x Reaction Buffer into a separate well (Assay Blank).

10. Add 5 µL of the 1xSucrose solution to each well. Tap plate to mix and incubate for 20 minutes at desired temperature.

11. Set up the Master Reaction Mix according to the scheme in Table 1. Prepare enough of the Master Reaction Mix for each of the sample, standard, and blank wells. The Master Reaction Mix should be prepared fresh each time the reaction is run.

Table1

	A	B
1	Reagent	Volume
2	Assay Buffer	95 µL
3	Enzyme Mix	1 µL
4	Dye Reagent	1 µL

12. Add 90 µL of the Master Reaction Mix to each of the blank, standard, and sample wells. Mix well using a horizontal shaker or by pipetting, and incubate the reaction for 20 minutes at room temperature. This is the enzyme reaction time. Protect the plate from light during the incubation.

13. For colorimetric assays, measure the absorbance at 570 nm (A570). For fluorometric assays, measure fluorescence intensity (FLU, **λ**ex = 535/ **λ**em = 587 nm).