Invertase Activity Assay Kit (Colorimetric)

Invertase Activity Assay Kit (Colorimetric) can be used to measure Invertase activity in biological and environmental samples.

Catalog number: ARG82171

Package: 100 tests

TABLE OF CONTENTS

MANUFACTURED BY:

Arigo Biolaboratories Corporation

Address: No. 22, Ln. 227, Gongyuan Rd., Hsinchu City 300, Taiwan

Phone: +886 (3) 562 1738

Fax: +886 (3) 561 3008

Email: info@arigobio.com

INTRODUCTION

Invertase is an enzyme that catalyzes the hydrolysis (breakdown) of sucrose into fructose and glucose. Alternative names for invertase include EC 3.2.1.26, saccharase, glucosucrase, beta-h-fructosidase, beta-fructosidase, invertin, sucrase, maxinvert L 1000, fructosylinvertase, alkaline invertase, acid invertase, and the systematic name: beta-fructofuranosidase. The resulting mixture of fructose and glucose is called inverted sugar syrup. Related to invertases are sucrases. Invertases and sucrases hydrolyze sucrose to give the same mixture of glucose and fructose. Invertases cleave the O-C (fructose) bond, whereas the sucrases cleave the O-C (glucose) bond. [Provide by Wikipedia: Invertase]

PRINCIPLE OF THE ASSAY

This Invertase Activity Assay Kit (Colorimetric) is a simple colorimetric assay that measures the activity of invertase in biological and environment samples. In the assay, invertase cleaves sucrose, resulting in the formation of fructose and glucose, which is determined by a colorimetric (O.D. 570 nm) or fluorimetric method (λex/em = 530/585 nm).

MATERIALS PROVIDED & STORAGE INFORMATION

The kit is shipped on ice. Store all components at -20°C upon receiving. Shelf life: 6 months after receipt.

Component	Quantity	Storage information
10X Reaction Buffer (pH 4.5)	12 mL	-20°C
Assay Buffer	10 mL	-20°C
10X Sucrose	1.5 mL	-20°C
Enzyme Mix	120 µL	-20°C
Dye Reagent	120 µL	-20°C
Standard (glucose)	1 mL	-20°C

MATERIALS REQUIRED BUT NOT PROVIDED

- Microplate reader capable of reading at O.D. 570 nm
- Fluorescence microplate reader capable of reading excitation at 530 nm and emission at 585 nm.
- Clear or black flat-bottom 96 well microplate
- Deionized or Distilled water
- Pipettes, pipette tips and Multichannel micropipette reservoir

TECHNICAL NOTES AND PRECAUTIONS

- Wear protective gloves, clothing, eye, and face protection especially while handling blood or body fluid samples.
- Interference: thiols (β-mercaptoethanol, dithioerythritol etc) at > 10 µM interfere with this assay and should be avoided. Glucose, if present in the sample, should be removed by dialysis or membrane filtration.
- Reagents are for research use only. Normal precautions for laboratory reagents should be exercised while using the reagents. Please refer to Material Safety Data Sheet for detailed information.
- All reagents should be mixed by gentle inversion or swirling prior to use. Do not induce foaming.
- Before using the kit, spin tubes and bring down all components to the bottom of tubes.
- It is highly recommended assaying the Standards and samples in duplicates.
- Change pipette tips between the addition of different reagent or samples.

SAMPLE COLLECTION & STORAGE INFORMATION

The sample collection and storage conditions listed below are intended as general guidelines. Sample stability has not been evaluated.

Environmental sample (E.g., soil): Weigh about 100 mg soil into a 1.5 mL Eppendorf tube. Add 880 µL of diluted Reaction Buffer and 120 µL of diluted sucrose. Mix thoroughly by homogenization and/or vortexing. Immediately transfer 200 µL of mixture into a clean tube and centrifuge for 2 minutes at 14,000 rpm. Transfer 100 µL clear supernatant into another clean tube and immediately freeze at-20°C. This "time zero" sample serves as a sample control. Incubate the invertase reaction for 1 hour at 30 or 37°C. Centrifuge for 2 minutes at 14,000 rpm. Transfer 40 µL clear supernatant and the above sample control for glucose determination.

Liquid biological sample: assay directly.

Note:

- Interference: thiols (β-mercaptoethanol, dithioerythritol etc) at > 10 µM interfere with this assay and should be avoided. Glucose, if present in the sample, should be removed by dialysis or membrane filtration.

REAGENT PREPARATION

- **1X Reaction Buffer:** Dilute the 10X Reaction Buffer to 1-fold by mixing 1 vol of the reagent and 9 vol of distilled water.
- **1X Sucrose:** Dilute the 10X Sucrose to 1-fold by mixing 1 vol of the reagent and 9 vol of distilled water.
- **Working Reagent:** for each well, mixing 1 µL of Enzyme Mix, 1 µL of Dye Reagent and 95 µL of Assay Buffer. Fresh reconstitution is recommended.
- **Standards:** Mix 5 µL of Standard with 828 µL of distilled water (final conc. 100 µM). Dilute Standards as follows;

Standard tube	Glucose (µM)	Distilled water (µL)	Standard Premix, 100 µM (µL)
S1	100	0	100
S2	50	40	60
S3	25	70	30
S4	0	100	0

ASSAY PROCEDURE

Equilibrate reagents to room temperature. Briefly centrifuge tubes before use.

	Standard wells	Sample wells	Control wells
Standards	40 µL		
Samples		40 µL	
1X Reaction Buffer			40 µL
1X Sucrose	5 µL	5 µL	5 µL
Tap plate to mix. Incubate for **20 minutes** at **30 or 37°C.**			
Working Reagent	90 µL	90 µL	90 µL
Tap plate to mix immediately. Incubate for **20 minutes** at **30 or 37°C** in the dark.			
Read the absorbance at O.D. 570 nm.			

Note:

- the procedure for fluorimetric assays is the same except that (1) a black flat-bottom 96-well plate is used, (2) glucose standards should be at 20, 12, 6 and 0 µM and that fluorescence intensity at λex/em = 530/585 nm is measured.
- Environmental sample like soil sample can be assayed as the protocol: SAMPLE COLLECTION & STORAGE INFORMATION.

CALCULATION OF RESULTS

1. Plot the glucose standard curve and determine its Slope (μM^{-1}). Invertase enzyme activity in the sample is calculated as follow:

   Invertase Activity (U/L) = $[(R_{Sample} - R_{Control}) / (Slope \times t)]$

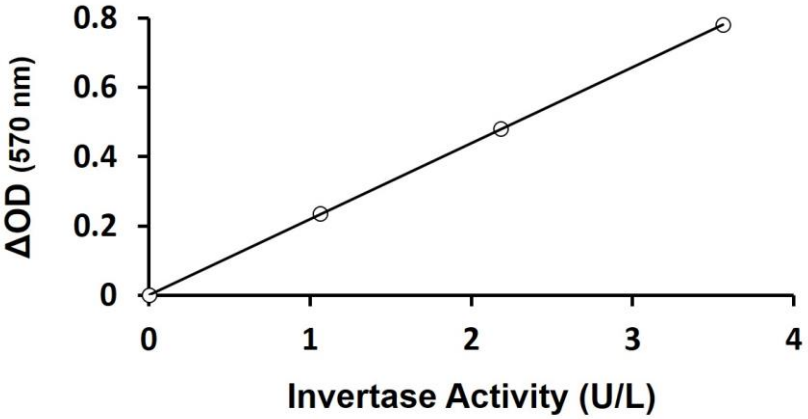
   Note:

   - R_{Sample}, $R_{Control}$: the O.D. 570 nm values or fluorescence intensity of the sample and sample control (1X Reaction Buffer).
   - t: the incubation time (20 minutes)

2. Unit definition: one unit of invertase catalyzes the formation of 1 µmole glucose per min at pH 4.5 under the assay conditions.
3. If the OD or fluorescence intensity is higher than the value for 100 µM glucose (colorimetric assay) or 20 µM (fluorimetric assay), dilute sample in 1X Reaction Buffer and repeat the assay. Multiply the result by the enzyme dilution factor.

EXAMPLE OF TYPICAL STANDARD CURVE

The following figures demonstrate typical results with the Invertase Activity Assay Kit (Colorimetric). One should use the data below for reference only. This data should not be used to interpret actual results.



QUALITY ASSURANCE

Sensitivity

0.007 U/L