

# Invertase Assay Kit (Fluorometric)

LS-K210-100 (100 Tests) • Store at -20°C



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## Introduction

INVERTASE ( $\beta$ -fructofuranosidase, EC 3.2.1.26) is an enzyme that catalyzes the hydrolysis of sucrose to fructose and glucose. Invertases cleave at the O-C(fructose) bond, whereas a related enzyme sucrase (EC 3.2.1.48) cleaves at the O-C(glucose) bond. A wide range of microorganisms produce invertase and can, thus, utilize sucrose as a nutrient. Invertase assay finds wide applications in environmental (e.g. soil), agricultural and food (confectionery) industry. LSBio's Invertase Assay Kit provides a convenient and ultra-sensitive colorimetric and fluorometric means to measure invertase activity. In the assay, invertase cleaves sucrose, resulting in the formation of fructose and glucose, which is determined by a colorimetric (570nm) or fluorometric method ( $\lambda_{em}/\lambda_{ex}$  = 585/530nm). The assay is simple, sensitive, stable and high-throughput adaptable.

## Key Features

- Safe. Non-radioactive assay.
- Sensitive and accurate. As low as 0.007 U/L invertase activity can be quantified.
- Homogeneous and convenient. "Mix-incubate-measure" type assay. No wash and reagent transfer steps are involved. Robust and amenable to HTS: can be readily automated on HTS liquid handling systems for processing thousands of samples per day.

## Applications

- Invertase and sucrase activity determination in biological and environmental (e.g. soil) samples.
- Evaluation and screening for invertase inhibitors.

## Components

Component	K210-100
	100 Tests
10x Reaction Buffer (pH 4.5)	12 mL
Assay Buffer	10 mL
Glucose Standard	1 mL
10x Sucrose	1.5 mL
Enzyme Mix	120 $\mu$ L
Dye Reagent	120 $\mu$ L

## Materials Not Supplied

Pipetting devices, centrifuge tubes, clear or black flat bottom 96-well plate (e.g. Corning Costar).

## Storage

The kit is shipped on ice. Store all kit components at -20 °C.

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## Assay Procedure

Interference: thiols ( $\beta$ -mercaptoethanol, dithioerythritol etc) at  $> 10 \mu\text{M}$  interfere with this assay and should be avoided. Glucose, if present in the sample, should be removed by dialysis or membrane filtration.

1. Assay Preparation. Prior to assay, bring all components to room temperature, briefly centrifuge tubes before opening. Dilute the provided 10x Reaction Buffer and 10x Sucrose to 1-fold by mixing 1 vol of the reagent with 9 vol of dH<sub>2</sub>O. Use the diluted reagents for all assays.

For glucose standard curve, mix 5  $\mu\text{L}$  Glucose Standard with 828  $\mu\text{L}$  dH<sub>2</sub>O (final 100  $\mu\text{M}$ ). Dilute as follows and transfer 40  $\mu\text{L}$  standards to separate wells in a clear flat-bottom 96-well plate.

No	100 $\mu\text{M}$ Std + H <sub>2</sub> O	Vol ( $\mu\text{L}$ )	Glucose ( $\mu\text{M}$ )
1	100 $\mu\text{L}$ + 0 $\mu\text{L}$	100	100
2	60 $\mu\text{L}$ + 40 $\mu\text{L}$	100	60
3	30 $\mu\text{L}$ + 70 $\mu\text{L}$	100	30
4	0 $\mu\text{L}$ + 100 $\mu\text{L}$	100	0

Sample: transfer 40  $\mu\text{L}$  sample to separate wells of the plate. As a sample control, use 40  $\mu\text{L}$  diluted Reaction Buffer.

2. Enzyme Reaction. Add 5  $\mu\text{L}$  of the diluted Sucrose to each well. Tap plate to mix. Incubate 20 min at desired temperature (e.g. 30°C).
3. Glucose Determination. Prepare enough Working Reagent in bulk. For each well, mix 95  $\mu\text{L}$  Assay Buffer, 1  $\mu\text{L}$  Enzyme Mix, 1  $\mu\text{L}$  Dye Reagent. Add 90  $\mu\text{L}$  Working Reagent to each well. Immediately tap plate to mix.

Incubate for 20 min in the dark. Read OD<sub>570nm</sub>.

*Note:* the procedure for fluorometric 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  $\mu\text{M}$  and that fluorescence intensity at  $\lambda_{em}/ex = 585/530\text{nm}$  is measured.

## Calculations

Plot glucose standard curve and determine its Slope ( $\mu\text{M}^{-1}$ ). Invertase enzyme activity in the sample is calculated as

$$\text{Invertase Activity} = \frac{R_{\text{SAMPLE}} - R_{\text{CONTROL}}}{\text{Slope} \times t} \quad (U/L)$$

where R<sub>SAMPLE</sub> and R<sub>CONTROL</sub> are the OD or fluorescence values of the sample and sample control (i.e. Reaction Buffer). t is the incubation time (20 min). Unit definition: one unit of invertase catalyzes the formation of 1  $\mu\text{mole}$  glucose per min at pH 4.5 under the assay conditions.

*Note:* if the OD or fluorescence intensity is higher than the value for 100  $\mu\text{M}$  glucose (colorimetric assay) or 20  $\mu\text{M}$  (fluorometric assay), dilute sample in 1-fold Reaction Buffer and repeat the assay. Multiply the result by the enzyme dilution factor.

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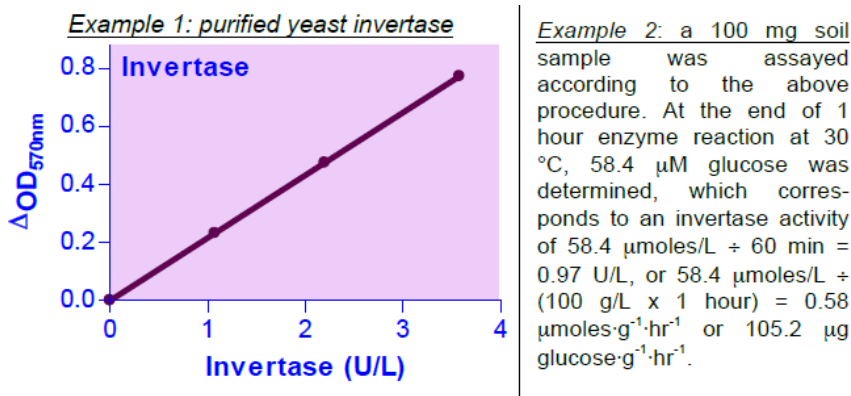
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## Invertase Assay in Soil Samples

Soil samples can be directly assayed as follows. Weigh about 100 mg soil into a 1.5-mL Eppendorf tube. Add 880  $\mu\text{L}$  diluted Reaction Buffer and 120  $\mu\text{L}$  diluted sucrose. Mix thoroughly by homogenization and/or vortexing. Immediately remove 200  $\mu\text{L}$  mixture into a clean tube and centrifuge for 2 min at 14,000 rpm. Transfer 100  $\mu\text{L}$  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 (Step 2). Centrifuge for 2 min at 14,000 rpm. Transfer 40  $\mu\text{L}$  clear supernatant and the above sample control for glucose determination (Step 3).

## Sample Data



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