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Product Information

Invertase Assay Kit

Catalog Number **MAK118**
Storage Temperature –20 °C

TECHNICAL BULLETIN

Product Description

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.

Components

The kit is sufficient for 100 assays in 96 well plates.

10× Reaction Buffer, pH 4.5 Catalog Number MAK118A	12 mL
Assay Buffer Catalog Number MAK118B	10 mL
Glucose Standard Catalog Number MAK118C	1 mL
10× Sucrose Catalog Number MAK118D	1.5 mL
Enzyme Mix Catalog Number MAK118E	120 μL
Dye Reagent Catalog Number MAK118F	120 μL

Reagents and Equipment Required but Not Provided.

- 96 well flat-bottom plate – It is recommended to use black plates with clear bottoms for fluorescence assays and clear plates for colorimetric assays.
- Fluorescence or spectrophotometric multiwell plate reader
- 10 kDa Molecular Weight Cut-Off (MWCO) Spin Filter

Precautions and Disclaimer

This product is for R&D use only, not for drug, household, or other uses. Please consult the Safety Data Sheet for information regarding hazards and safe handling practices.

Storage/Stability

The kit is shipped on dry ice. If desired, the Reaction Buffer and the Assay Buffer can be stored at 2-8 °C. Store all other reagents at –20 °C.

Procedure

Bring all reagents to room temperature prior to use. Briefly centrifuge vials before opening. Use ultrapure water for the preparation of reagents. To maintain reagent integrity, avoid repeated freeze/thaw cycles.

Prior to assay, dilute the 10× Reaction Buffer and the 10× Sucrose solution to 1× by mixing one volume of each 10× solution with 9 volumes of water. The 1× solutions should be used in the assays.

This assay can be performed at either 30 °C or 37 °C.

Sample Preparation

Note: Thiols, such as β-mercaptoethanol or dithioerythritol, at >10 μM can interfere with this assay and should be avoided during sampler preparation. The presence of glucose in samples will contribute to background in this assay. Glucose, if present in the sample, can be removed by dialysis or membrane filtration.

Soil Samples can be prepared and assayed as follows. Weigh 100 mg of soil into a 1.5 mL microcentrifuge tube. Add 880 μL of 1× Reaction Buffer and 120 μL of 1× sucrose solution. Mix thoroughly by homogenization and/or vortexing. Immediately remove 200 μL of the mixture into a clean tube and centrifuge for 2 minutes at 14,000 rpm. Transfer 100 μL of the cleared supernatant into another clean tube and immediately freeze at –20 °C. This sample will serve as the sample control. Incubate the remaining 100 μL of the reaction mixture for 1 hour at either 30 °C or 37 °C. Set up standards as indicated below. Following 1 hour sample incubation, centrifuge for 2 minutes at 14,000 rpm. Transfer 40 μL of clarified soil samples (sample control and sample) to plate. Continue with procedure at step 3 of Assay Reaction.

Glucose Standards for Colorimetric Detection

Dilute 5 μL of the Glucose Standard with 828 μL of water to create a 100 μM Glucose Solution. Dilute the 100 μM Glucose Solution as indicated in Table 1.

Table 1.
Colorimetric Standards

Number	Standard	Water	Glucose Concentration
1	100 μL	0 μL	100 μM
2	60 μL	40 μL	60 μM
3	30 μL	70 μL	30 μM
4	0 μL	100 μL	0 μM

Glucose Standards for Fluorometric Detection

Dilute 5 μL of the Glucose Standard with 828 μL of water to create a 100 μM Glucose Solution. Dilute the 100 μM Glucose Solution as indicated in Table 2.

Table 2.
Fluorometric Standards

Number	Standard	Water	Glucose Concentration
1	20 μL	80 μL	20 μM
2	12 μL	88 μL	12 μM
3	6 μL	94 μL	6 μM
4	0 μL	100 μL	0 μM

Assay Reaction

1. Transfer 40 μL of the appropriate standards and 40 μL of samples into separate wells of a 96 well plate. Transfer 40 μL of diluted 1× Reaction Buffer into a separate well (Assay Blank).

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

3. Set up the Master Reaction Mix according to the scheme in Table 3. 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.

Table 3.
Master Reaction Mix

Reagent	Volume
Assay Buffer	95 μL
Enzyme Mix	1 μL
Dye Reagent	1 μL

4. 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.

5. For colorimetric assays, measure the absorbance at 570 nm (A_{570}). For fluorometric assays, measure fluorescence intensity (FLU, λ_{ex} = 535/ λ_{em} = 587 nm).
   Note: If the sample absorbance is higher than the standard absorbance at 100 μM (for the colorimetric assay) or 20 μM (for the fluorometric assay), dilute the sample in 1× Reaction Buffer and repeat the assay. Multiply the results by the dilution factor.

Results

Calculations

Use the values obtained from the appropriate glucose standards to plot a standard curve and determine the slope using linear regression fitting.

Note: A new standard curve must be set up each time the assay is run.

Invertase Activity

The amount of Invertase enzymatic activity in the sample can be calculated using the equations:

$$\text{Invertase Activity (units/L)} = \frac{R_{sample} - R_{blank}}{\text{Slope} \times t}$$

where:

R_{sample} = Absorbance or FLU of sample

R_{blank} = Absorbance or FLU of assay blank (reaction buffer)

t = Enzyme Reaction time (20 minutes)

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.

Troubleshooting Guide

Problem	Possible Cause	Suggested Solution
Assay not working	Cold assay buffer	Assay Buffer must be at temperature indicated in bulletin
	Omission of step in procedure	Refer and follow Technical Bulletin precisely
	Plate reader at incorrect wavelength	Check filter settings of instrument
	Type of 96 well plate used	For fluorescence assays, use black plates with clear bottoms. For colorimetric assays, use clear plates
Lower/higher readings in samples and standards	Improperly thawed components	Thaw all components completely and mix gently before use
	Incorrect incubation times or temperatures	Refer to Technical Bulletin and verify correct incubation times and temperatures
	Incorrect volumes used	Use calibrated pipettes and aliquot correctly
Non-linear standard curve	Use of partially thawed components	Thaw and resuspend all components before preparing the reaction mix
	Pipetting errors in preparation of standards	Avoid pipetting small volumes
	Pipetting errors in the Reaction Mix	Prepare a master Reaction Mix whenever possible
	Air bubbles formed in well	Pipette gently against the wall of the plate well
	Standard stock is at incorrect concentration	Refer to the standard dilution instructions in the Technical Bulletin
	Calculation errors	Recheck calculations after referring to Technical Bulletin
	Substituting reagents from older kits/lots	Use fresh components from the same kit
Unanticipated results	Samples measured at incorrect wavelength	Check the equipment and filter settings
	Samples contain interfering substances	If possible, dilute sample further
	Sample readings above/below the linear range	Concentrate or dilute samples so readings are in the linear range

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