Technical Manual

ColorFluor Invertase Activity Assay Kit

Catalogue Code: BA0123

Pack Size: 100 assays

Research Use Only

DESCRIPTION

INVERTASE (β-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.

The Assay Genie ColorFluor Invertase Activity Assay Kit provides a convenient and ultra-sensitive colorimetric and fluorimetric 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 fluorimetric method ($l_{em/ex}$ = 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.

KIT CONTENTS

10x Reaction Buffer:	12 mL (pH 4.5)	10x Sucrose:	1.5 mL
Assay Buffer:	10 mL	Enzyme Mix:	120 µL
Glucose Standard:	1 mL	Dye Reagent:	120 µL

Storage conditions. This product is shipped on ice. Store Reaction Buffer and Assay Buffer at 4°C and other reagents at -20°C. Shelf life of six months after receipt.

Precautions: 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.

ASSAY PROCEDURE

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.

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_2O. Use the diluted reagents for all assays.

   For glucose standard curve, mix 5 µL Glucose Standard with 828 µL dH_2O (final 100 µM). Dilute as follows and transfer 40 µL standards to separate wells in a clear flat-bottom 96-well plate.

No	100 µM Std + H_2O	Vol (µL)	Glucose (µM)
1	100 µL + 0 µL	100	100
2	60 µL + 40 µL	100	60
3	30 µL + 70 µL	100	30
4	0 µL + 100 µL	100	0

Sample: transfer 40 µL sample to separate wells of the plate. As a sample control, use 40 µL diluted Reaction Buffer.

2. *Enzyme Reaction*. Add 5 µL 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 µL Assay Buffer, 1 µL Enzyme Mix, 1 µL Dye Reagent. Add 90 µL Working Reagent to each well. Immediately tap plate to mix.

   Incubate for 20 min in the dark. Read OD570nm.

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 $I_{em/ex}$ = 585/530nm is measured.

CALCULATION

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

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

where R_{SAMPLE} and $R_{CONTROL}$ 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 µmole 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 µM glucose (colorimetric assay) or 20 µM (fluorimetric assay), dilute sample in 1-fold Reaction Buffer and repeat the assay. Multiply the result by the enzyme dilution factor.

INVERTASE ASSAY IN SOIL SAMPLES

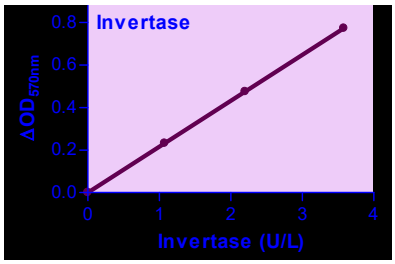
Soil samples can be directly assayed as follows. Weigh about 100 mg soil into a 1.5-mL Eppendorf tube. Add 880 µL diluted Reaction Buffer and 120 µL diluted sucrose. Mix thoroughly by homogenization and/or vortexing. Immediately remove 200 µL mixture into a clean tube and centrifuge for 2 min at 14,000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

MATERIALS REQUIRED, BUT NOT PROVIDED

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

Example 1: purified yeast invertase



***EXAMPLE 2*:** a 100 mg soil sample was assayed according to the above procedure. at the end of 1 hour enzyme reaction at 30 °c, 58.4 µm glucose was determined, which corresponds to an invertase activity of 58.4 µmoles/l ÷ 60 min = 0.97 u/l, or 58.4 µmoles/l ÷ (100 g/l x 1 hour) = 0.58 µmoles·g^{-1}·hr^{-1} or 105.2 µg glucose·g^{-1}·hr^{-1}.

LITERATURE

1. Huang, Y.H., Picha, D.H. and Johnson, C.E.(1998). An alternative method for enzymatic assay of plant invertases. J. Agric. Food Chem. 46 (8): 3158–3161.
2. Ewing, E.E. and McAdoo, M.H. (1971). An examination of methods used to assay potato tuber invertase and its naturally occurring inhibitor. Plant Physiol. 48: 366-370.
3. Silveira, M.C., Carvajal, E. and Bon, E.P. (1996). Assay for in vivo yeast invertase activity using NaF. Anal Biochem. 238: 26-28.

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