

## EnzyChrom™ Invertase Assay Kit (EIVT-100)

### Quantitative Determination of Invertase/Sucrase Activity

#### DESCRIPTION

**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.

BioAssay Systems' Invertase 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 ( $\lambda_{em/ex}$  = 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

<b>10x Reaction Buffer:</b> 12 mL (pH 4.5)	<b>10x Sucrose:</b> 1.5 mL
<b>Assay Buffer:</b> 10 mL	<b>Enzyme Mix:</b> 120 $\mu$ L
<b>Glucose Standard:</b> 1 mL	<b>Dye Reagent:</b> 120 $\mu$ L

**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 ( $\beta$ -mercaptoethanol, dithioerythritol etc) at > 10  $\mu$ 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  $\mu$ L Glucose Standard with 828  $\mu$ L dH<sub>2</sub>O (final 100  $\mu$ M). Dilute as follows and transfer 40  $\mu$ L standards

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

to separate wells in a clear flat-bottom 96-well plate.

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

2. **Enzyme Reaction.** Add 5  $\mu$ 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  $\mu$ L Assay Buffer, 1  $\mu$ L Enzyme Mix, 1  $\mu$ L Dye Reagent. Add 90  $\mu$ 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 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  $\mu$ M and that fluorescence intensity at  $\lambda_{em/ex}$  = 585/530nm is measured.

#### CALCULATION

Plot glucose standard curve and determine its Slope ( $\mu$ M<sup>-1</sup>). Invertase enzyme activity in the sample is calculated as

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

where  $R_{\text{SAMPLE}}$  and  $R_{\text{CONTROL}}$  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  $\mu$ 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  $\mu$ M glucose (colorimetric assay) or 20  $\mu$ M (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

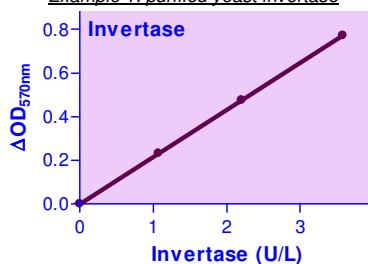
Soil samples can be directly assayed as follows. Weigh about 100 mg soil into a 1.5-mL Eppendorf tube. Add 880  $\mu$ L diluted Reaction Buffer and 120  $\mu$ L diluted sucrose. Mix thoroughly by homogenization and/or vortexing. Immediately remove 200  $\mu$ L mixture into a clean tube and centrifuge for 2 min at 14,000 rpm. Transfer 100  $\mu$ 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  $\mu$ L 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  $\mu$ M glucose was determined, which corresponds to an invertase activity of 58.4  $\mu$ moles/L  $\div$  60 min = 0.97 U/L, or 58.4  $\mu$ moles/L  $\div$  (100 g/L  $\times$  1 hour) = 0.58  $\mu$ moles-g<sup>-1</sup>·hr<sup>-1</sup> or 105.2  $\mu$ g glucose-g<sup>-1</sup>·hr<sup>-1</sup>.*

#### LITERATURE

- 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.
- 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.
- Silveira, M.C., Carvajal, E. and Bon, E.P. (1996). Assay for in vivo yeast invertase activity using NaF. *Anal Biochem.* 238: 26-28.