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Invertase Activity Colorimetric Assay Kit

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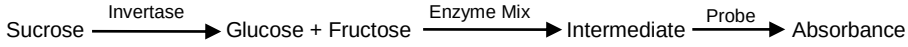
(Catalog # K674-100; 100 assays; Store at -20°C)

I. Introduction:

Invertase (EC 3.2.1.26), also known as sucrase or β-fructofuranosidase, catalyzes the hydrolysis of sucrose (table sugar) by cleaving its glycosidic bond and forming one molecule each of glucose and fructose. Invertase is widely expressed among plants and microorganisms including yeast and bacteria. It is extensively utilized in industrial processes, including food, ethanol production, cosmetics, drugs, paper and bioelectronics electrodes. BioVision's Invertase Activity Assay kit provides a simple, sensitive and high-throughput adaptable assay that detects relevant concentrations of Invertase in biological samples or purified/crude enzyme preparations. The assay is based on the enzymatic detection of glucose, one of the hydrolysis products. Detection limit: 20 µU.



II. Application:

- Measurement of Invertase activity in various biological samples/preparations

III. Sample Type:

- Biological fluids e.g. saliva
- Bacteria
- Crude/purified enzyme preparations

IV. Kit Contents:

Components	K674-100	Cap Code	Part Number
Invertase Hydrolysis Buffer	25 ml	NM	K674-100-1
Invertase Assay Buffer	25 ml	WM	K674-100-2
Invertase Stop Solution	1 ml	Brown	K674-100-3
OxiRed™ Probe (in DMSO)	0.2 ml	Red	K674-100-4
Invertase Enzyme Mix	Lyophilized	Green	K674-100-5
Invertase Substrate	1.2 ml	Blue	K674-100-6
Glucose Standard (100 mM)	100 µl	Yellow	K674-100-7
Positive Control	Lyophilized	Orange	K674-100-8

V. User Supplied Reagents and Equipment:

- 96-well clear plate with flat bottom
- Multi-well spectrophotometer
- 10 kDa Spin Column (Cat # 1997)

VI. Storage Conditions and Reagent Preparation:

Store kit at -20°C, protected from light. Briefly spin small vials prior to opening. Read entire protocol before performing the assay.

- **Invertase Hydrolysis Buffer and Invertase Assay Buffer**: Bring to room temperature (RT) before use. Store at -20°C.
- **OxiRed™ Probe (in DMSO):** Protect from light. Warm to RT before use. Store at -20°C.
- **Invertase Enzyme Mix:** Reconstitute with 220 µl Invertase Assay Buffer. Pipette gently to dissolve. Aliquot and store at -20°C. Keep on ice while in use. Use within two months.
- **Invertase Substrate:** Ready to use as supplied. Store at -20°C. Keep on ice while in use.
- **Glucose Standard:** Bring to RT before use.
- **Positive Control:** Reconstitute with 140 µl Invertase Hydrolysis Buffer. Mix well. Aliquot and store at -20°C. Avoid repeated freeze/thaw cycles. Use within two months.

VII. Invertase Activity Assay Protocol:

1. **Sample Preparation:** For bacterial samples: collect cells by centrifugation (10,000 X g, 10 min.) in a pre-weighed centrifuge tube. Remove supernatant and determine wet weight of the pellet. Resuspend the pellet in Invertase Hydrolysis Buffer using 5 µl of Hydrolysis Buffer per mg of sample. Sonicate samples on ice for 1 min. and keep on ice for 5 min., repeat this cycle three times. Centrifuge samples (10,000 X g, 20 min., 4°C) and collect the supernatant. Add 1-40 µl of supernatant into desired well(s) in a 96-well plate. Adjust the volume to 40 µl/well with Hydrolysis Buffer. For biological fluids such as saliva, centrifuge at 10,000 X g for 5 min. at 4°C. Collect supernatant and add 1-40 µl into desired well(s) in a 96-well plate. Adjust the volume to 40 µl/well with Invertase Hydrolysis Buffer. Add 2-10 µl of diluted Positive Control into desired well(s). Adjust the volume to 40 µl/well with Hydrolysis Buffer.

   Notes:

   a. Glucose in sample will contribute significantly to the background signal. If interference is expected in the sample, prepare parallel sample well(s) as sample background control(s). Make up the volume to 50 µl/well with Hydrolysis Buffer.

   b. For unknown samples, we recommend doing a pilot experiment & testing several doses to ensure the readings are within the Standard Curve range.

2. **Standard Curve Preparation:** Prepare 1 mM Glucose Standard by adding 10 µl of 100 mM Glucose Standard into 990 µl of ddH_2O. Add 0, 2, 4, 6, 8, and 10 µl of 1 mM Glucose Standard into a series of wells in a 96-well plate to generate 0, 2, 4, 6, 8 and 10 nmol of glucose/well. Adjust the volume to 50 µl/well with Hydrolysis Buffer.
3. **Substrate Hydrolysis:** Add 10 µl Invertase Substrate into each well containing samples and Positive Control. Mix well. Incubate for 1-20 min. at 37°C. Record incubation time and then add 10 µl of Invertase Stop Solution into each well containing samples, Positive Control, Standards, and background control(s). Mix well.

   Note:

   Incubation time depends on the Invertase activity in samples. Longer incubation times may be required for samples having low Invertase activity.
4. **Reaction Mix:** Mix enough reagents for the total number of wells to be assayed including Standards, samples, Positive Control and background control(s). For each well, prepare 40 µl of Reaction Mix containing:

	Reaction Mix
Invertase Assay Buffer	36 µl
Invertase Enzyme Mix	2 µl
OxiRed™ Probe	2 µl

   Mix well. Add 40 µl of Reaction Mix into each well. Mix.
5. **Measurement:** Incubate plate at 37°C for 30 min., protected from light. Measure absorbance (570 nm).
6. **Calculation:** Subtract 0 Standard reading from all readings. Plot the Glucose Standard Curve. If sample background control is significant, then subtract sample background control reading from sample reading. Apply ΔOD to Standard Curve to get B nmol of glucose generated by Invertase during substrate hydrolysis (1-20 min.).

Sample Invertase Activity = BXD/(TXV) nmol/min.ml or mU/ml

Where: **B** is amount of glucose from Standard Curve (nmol)
T is incubation time for substrate hydrolysis (min.)
V is sample volume added into the reaction well (ml)
D is sample dilution factor

Unit Definition: One unit of Invertase activity is the amount of enzyme that generates 1.0 µmol of Glucose per min. at pH 4.5 at 37 °C.

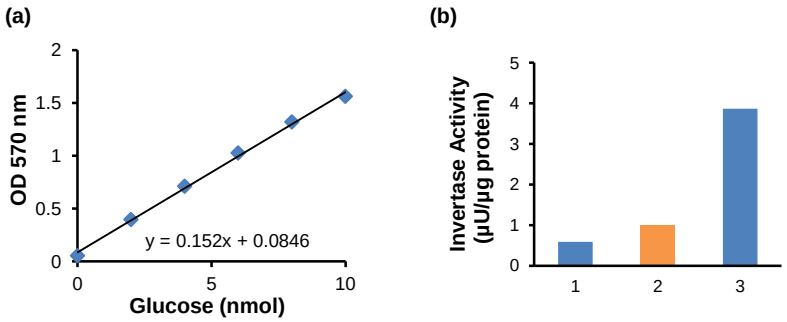


Figure: (a) Glucose Standard Curve. **(b)** Detection of bacterial Invertase activity in human saliva. Samples collected: 1- Fasting; 2- Low calorie meal and 3: High Sucrose meal. Undiluted samples (1 and 2 = 25 µl and 3 = 10 µl) were incubated for 20 min. with Invertase substrate. Assay was performed following the kit protocol.

VIII. Related Products:

Glucose Colorimetric/Fluorometric Assay Kit (K606)
Glucose Colorimetric Assay Kit II (K686)
Galactose and Lactose Colorimetric/Fluorometric Assay Kit (K617)
Fructose Colorimetric/Fluorometric Assay Kit (K619)
Maltose Colorimetric/Fluorometric Assay Kit (K628)
Glucose and Sucrose Colorimetric/Fluorometric Assay Kit (K616)
PicoProbe™ Glucose Fluorometric Assay Kit (K688)
Maltose and Glucose Colorimetric/Fluorometric Assay Kit (K618)
Galactose Colorimetric/Fluorometric Assay Kit (K621)

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