Sucrose Synthase Assay Kit

Sucrose Synthase Assay Kit is a detection kit for the quantification of Sucrose Synthase Activity in tissue extracts and cell lysate.

Catalog number: ARG82022

Package: 96 wells

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INTRODUCTION

In enzymology, a sucrose synthase (EC 2.4.1.13) is an enzyme that catalyzes the chemical reaction.

NDP-glucose + D-fructose ⇌ NDP + sucrose

Thus, the two substrates of this enzyme are NDP-glucose and D-fructose, whereas its two products are NDP and sucrose.

This enzyme belongs to the family of glycosyltransferases, specifically the hexosyltransferases. The systematic name of this enzyme class is NDP-glucose: D-fructose 2-alpha-D-glucosyltransferase. This enzyme participates in starch and sucrose metabolism. [Provide by Wikipedia: Sucrose synthase]

PRINCIPLE OF THE ASSAY

This Sucrose synthase Assay Kit is a simple colorimetric assay that measures the amount of Sucrose synthase activity in tissue extracts and cell lysate. The assay is based on the enzyme driven reaction. SUS catalyze the UDPG reaction of free fructose and glucose to generate sucrose, and then react with resorcinol present a color change, have a characteristic absorption peak at O.D. 480 nm. The concentration of Sucrose synthase in the samples is then determined by comparing the O.D. 480 nm absorbance of samples to the standard curve.

MATERIALS PROVIDED & STORAGE INFORMATION

Component	Quantity	Storage information
96 Well microplate	1 plate	RT
Assay Buffer	4 x 30 mL (ready to use)	4°C
Substrate (lyophilized)	1 vial	4°C
Substrate Diluent	3 mL (ready to use)	4°C
Reaction Buffer	10 mL	4°C
Stop Solution	1 mL	4°C
Dye Reagent (lyophilized)	1 vial	4°C
Standards (lyophilized)	1 vial	4°C
Technical Manual	1 ea	RT

MATERIALS REQUIRED BUT NOT PROVIDED

- Microplate reader capable of reading at 480 nm
- Centrifuge
- Mortar
- Deionized or Distilled water
- Ice
- Pipettes and pipette tips
- Multichannel micropipette reservoir

TECHNICAL NOTES AND PRECAUTIONS

- Wear protective gloves, clothing, eye, and face protection especially while handling blood or body fluid samples.
- The concentrations can vary over a wide range depending on the different samples. For unknown samples, we recommend doing a pilot experiment & testing several doses to ensure the readings are within the standard curve range.
- All reagents should be mixed by gentle inversion or swirling prior to use. Do not induce foaming.
- Before using the kit, spin tubes and bring down all components to the bottom of tubes.
- It is highly recommended assaying the Standards and samples in duplicates.
- Change pipette tips between the addition of different reagent or samples.

SAMPLE COLLECTION & STORAGE INFORMATION

The sample collection and storage conditions listed below are intended as general guidelines. Sample stability has not been evaluated.

Tissue samples: Weigh out 0.1 g tissue, homogenize with 1 mL of Assay Buffer on ice, centrifuged at 8,000 x g for 10 minutes at 4°C. Take the supernatant into a new centrifuge tube and keep it on ice for detection.

Liquid samples: detect it directly, or dilute with Assay Buffer.

REAGENT PREPARATION

- Substrate: Add 3 mL of Substrate Diluent to dissolve before use.
- Dye Reagent: Add 5 mL of distilled water to dissolve before use.
- Standards: Add 1 mL of distilled water to dissolve before use. The concentration will be 4 mg/mL. Use the 4 mg/mL Standards to prepare a series of diluted standards according to the Table below.

Standard tube	Final Standard conc. (mg/mL)	distilled water (μL)	Volume of 4 mg/mL Standards (μL)
S1	4	0	500
S2	2	250	250 of S1
S3	1	250	250 of S2
S4	0.5	250	250 of S3
S5	0.25	250	250 of S4
S6	0.125	250	250 of S5

ASSAY PROCEDURE

Each Standard and sample should be assayed in duplicate or triplicate. A freshly prepared standard curve should be used each time the assay is performed.

Add following reagents into the microcentrifuge tubes:			
Reagent	**Sample**	**Standards**	**Blank**
Sample	10 µL		
Standards		10 µL	
Distilled water			10 µL
Substrate	30 µL	30 µL	30 µL
Mix well and incubate for **10 minutes** at **30°C**			
Stop Solution	10 µL	10 µL	10 µL
Mix well and incubate for **10 minutes** in the **boiling water bath**, then put them in ice.			
Reaction Buffer	100 µL	100 µL	100 µL
Dye Reagent	50 µL	50 µL	50 µL
Mix well, and incubate for **5 minutes** in the **boiling water bath**. Centrifuge and transfer all reagents to the **96 Well microplate**. Read the absorbance at **O.D. 480 nm.**			

CALCULATION OF RESULTS

1. Calculate the average absorbance value for each set of Standards, Control, Blank and samples.
2. Using linear graph paper, construct a standard curve by plotting the mean absorbance value obtained from each standard against its concentration with absorbance value on the vertical (Y) axis and concentration on the horizontal (X) axis.
3. Use the mean absorbance value for each sample determine the corresponding concentration from the standard curve.
4. Unit Definition: One unit of SUS activity is defined as the enzyme generates 1 µg of sucrose per minute.

5. According to the weight of sample:

SUS (U/g)

$= \{[(C_{Standaard} \times V_{Standardl}) \times (OD_{Sample} - OD_{Blank}) / (OD_{Standard} - OD_{Blank})] / (V_{Sample} \times W / V_{Assay})\} / T$

$= [200 \times (OD_{Sample} - OD_{Blank}) / (OD_{Standard} - OD_{Blank})] / W$

6. According to the protein concentration of sample:

SUS (U/mg)

$= \{[(C_{Standaard} \times V_{Standardl}) \times (OD_{Sample} - OD_{Blank}) / (OD_{Standard} - OD_{Blank})] / (V_{Sample} \times C_{Protein})\} / T$

$= [200 \times (OD_{Sample} - OD_{Blank}) / (OD_{Standard} - OD_{Blank})] / C_{Protein}$

Note:

W: the weight of sample, g;

$C_{Standard}$: the concentration of standard, 4 mg/mL = 4000 μg/mL;

$C_{Protein}$: the protein concentration, mg/mL;

V_{Assay}: the volume of Assay Buffer, 1 mL;

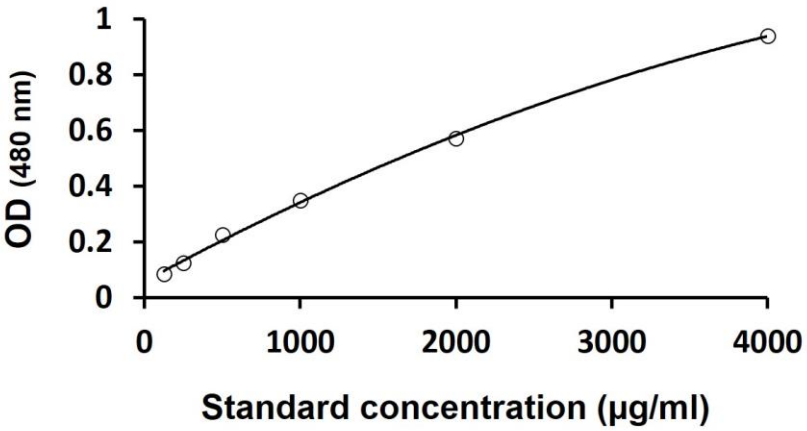
V_{Sample}: the volume of sample, 0.01 mL;

$V_{standard}$: the volume of Standard, 0.01 mL;

T: the reaction time, 10 minutes.

EXAMPLE OF TYPICAL STANDARD CURVE

The following figures demonstrate typical results with the Sucrose Synthase Assay Kit. One should use the data below for reference only. This data should not be used to interpret actual results.



QUALITY ASSURANCE

Sensitivity

100 µg/mL