Sucrose Microplate Assay Kit

Instructions for Use | Cat. No.: ARS6644

Detection and Quantification of Sucrose Content in Tissue extracts, Cell lysate Samples.

For research use only . Not for diagnostic or therapeutic procedures .

1. Introduction

Sucrose (C12H22O11) is a disaccharide of glucose and fructose with an α -1,2-glycosidic linkage. It is the most common food sweetener and the most important sugar in plants.

Sucrose Colorimetric Microplate Assay Kit provides a convenient means tomeasure sucrose concentration in biological samples. In the assay,sucrase cleaves sucrose, resulting in the formation of fructose and glucose. The enzyme catalysed reaction products can be measured at a colorimetric readout at 540 nm.

2. Kit Components

Component	Volume	Storage
96-Well Microplate	1 plate	-
Assay Buffer	30 mlx 4	4 °C
Enzyme	Powder x 1	-20 °C
Reaction Buffer	12 mlx 1	4 °C
Dye Reagent	20 mlx 1	4 °C, keep in dark
Standard	Powder x 1	4 °C
Plate Adhesive Strips	3 strips	-
Technical Manual	1 Manual	-

3. Materials Required But Not Provided

1. Microplate reader to read absorbance at 540 nm
2. Distilled water
3. Pipettor , multi-channel pipettor
4. Pipette tips
5. Mortar
6. Centrifuge
7. Timer
8. Hot Air Circulation Oven

4. Reagent Preparation

Standard: Briefly centrifuge prior to opening. Dissolve in 1 mL distilled water to generate 10 mmol/ L of standardtop solution , store at 4°C for 2-3 month safter reconstitution. Perform 2 -fold serial dilutions of the top standardsolution using distilled water to make the standard curve.The concentration of standard curve could be 10/5/2.5/1.25/0.625/0.312/0.156 mmol/L.

Enzyme: Briefly centrifuge prior to opening.Add 1 mL Reaction Buffer to dissolve before use. Store at 4°C for 1 week or -20°C for 1 month after reconstitution.

Note: Divide into small aliquots to avoid repeated freeze-thaw cycles.

5. Sample Preparation

1. For tissue samples Weigh out 0.1g tissue, homogenize with 1 mL Assay buffer on ice, put it in water bath of 80°C for 1 0 minutes ,centrifuged at 4 ,000g at room temperature for 10minutes, take the supernatant into a new centrifuge tube.

2. For liquid samples Detect directly.

6. Assay Procedure

Add following reagents in to the microcentrifuge tubes:

Reagent*	Sample**	Control	Standard	Blank
Sample	40 µl	40 µl	--	--
Standard	--	--	40 µl	--
Distilled water	--	--	--	40 µl
Reaction Buffer	50 µl	60 µl	50 µl	50 µl
Enzyme	10 µl	--	10 µl	10 µl
Mix,put it in the oven,37°C for 20 minutes.				
Dye Reagent	100 µl	100 µl	100 µl	100 µl
Mix, put it into the convection oven,90 °Cfor 10 minutes, record absorbance measured at 540nm.				

Note:

*Reagents must be added sequentially and should not be premixed prior to addition.

**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.

7. Calculation

1. Calculate the sample concentration in ASSAY PROCEDURE according to the slope of

the standard curve

$$C = \frac{(OD_{Sample} - OD_{Blank}) - Intercept}{Slope} \times n \ (mmol/L)$$

Calculate the initial concentration according to sample preparation procedure .

2. According to one point of the standard OD and concentration

2.1 According to the protein concentration of sample

$$C = \frac{(C_{Standard} \times V_{Standard}) \times (OD_{Sample} - OD_{Blank})}{(OD_{Standard} - OD_{Blank}) \times C_{Protein} \times V_{Sample}} \ (\mu mol/mg)$$

2.2According to the weight of sample

$$C = \frac{(C_{Standard} \times V_{Standard}) \times (OD_{Sample} - OD_{Blank})}{(OD_{Standard} - OD_{Blank}) \times W \times (V_{Sample} / V_{Assay})} \ (\mu mol/g)$$

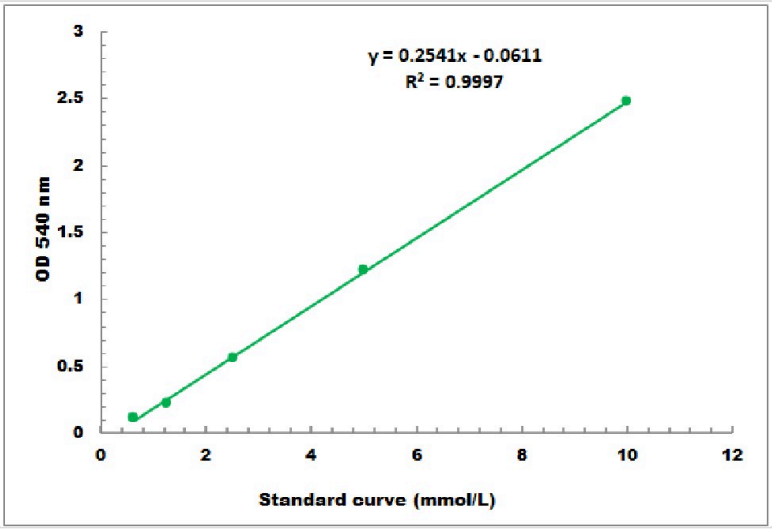
2.3According to the volume of sample

$$C = \frac{(C_{Standard} \times V_{Standard}) \times (OD_{Sample} - OD_{Blank})}{(OD_{Standard} - OD_{Blank}) \times V_{Sample}} \ (\mu mol/ml)$$

Item	Definition
Slope	the absorbance slope of standard curve
n	the dilution factor
$C_{Standard}$	the standard concentration, mmol/L = μmol/ml
$V_{Standard}$	the volume of standard in assay procedure, μl
V_{Sample}	the volume of sample in assay procedure, μl
V_{Assay}	the volume of Assay Buffer, μl
$C_{Protein}$	the sample protein concentration, mg/ml
W	the weight of sample, g

8. Typical Data

The standard curve is for demonstration only. A standard curve must be run with each assay.



Detection Range : 0.5mmol/ L -10mmol/ L