Sucrase Activity Microplate Assay Kit

Instructions for Use | Cat. No.: ARS6645

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

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

1. Introduction

Sucrase is the name given to a number of enzymes located in on the brush border of the small intestine that catalyze the hydrol ysis of sucrose to fructose and glucose.

The enzyme invertase, which occurs more commonly in plants, also hydrolyzes sucrose but by a different mechanism.

Sucrase Activity Colorimetric Microplate Assay Kit provides a convenient means tomeasure sucrase activity 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
Substrate	4 mlx 1	4 °C
Stop Solution	2 mlx 1	4 °C
Dye Reagent	10 mlx 1	4 °C
Standard	Powderx 1	4 °C
Positive Control	Powderx 1	-20 °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. Ice
9. Convection oven

4. Reagent Preparation

Standard: Briefly centrifuge prior to opening. Dissolve in 1 mL distilled water to generate 20 mmol/ L of standard top solution.Store at 4°C for 1 month or -20°C for 6-7 months after 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 20/10/5/2.5/1.25/0.625/0.312mmol/L.

Positive Control: Briefly centrifuge prior to opening. Add 1 mL distilled water to dissolve before use. Store at -80°C for 1 month after reconstitution.

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

5. Sample Preparation

1. For cell and bacteria samples Collect cell or bacteria into centrifuge tube, discard the supernatant after centrifugation, add 1 mL Assay Buffer for 5 × 10^6 cell or bacteria, sonicate (with power 20%, sonicate 3s, interval 10s ,repeat 30 times); centrifuged at 8000 g 4°C for 10minutes , take the supernatant into a new centrifuge tube and keep it on ice for detection.

2. For tissue samples Weigh out 0.1 g tissue, homogenize with 1 mL Assay Buffer on ice, centrifuged at 8000 g 4°C for 10minutes , take the supernatant into a new centrifuge tube and keep it on ice for detection.

3. For liquid samples Detect directly.

6. Assay Procedure

Add following reagents into the microplate:

Reagent*	Sample**	Control	Standard	Blank	Positive Control
Sample	40 μL	--	--	--	40 μL
Stop Solution	--	20 μL	--	--	--
Substrate	40 μL	40 μL	--	--	40 μL
Mix, put it in the oven, 37°C for 5 minutes.					
Stop Solution	20 μL	--	--	--	20 μL
Standard	--	--	40 μL	--	--
Distilled water	--	40 μL	60 μL	100 μL	--
Dye Reagent	100 μL	100 μL	100 μL	100 μL	100 μL
Mix, put it into the convection oven, 90°C for 10 minutes, record absorbance measured at 540nm.					

Note:

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

**For unknown samples, we recommend doing a pilot experiment & testing several doses to ensure the readings are within the standard curve range. If the enzyme activity is lower, please add more samples into the reaction system; or increase the reaction time; if the enzyme activity is higher, please dilute the sample, or decrease the reaction time.

7. Calculation

Unit Definition: One unit of Sucrase activity is the enzyme that generates 1μmol of reducing sugars per minute.

1. According to the slope of the standard curve

$$\text{Activity} = \frac{(OD_{Sample}-OD_{Control}) - \text{Intercept}}{\text{Slope} \times T} \times n \ (U/mL)$$

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

2.1 According to the protein concentration of sample

$$\text{Activity} = \frac{(C_{Standard} \times V_{Standard}) \times (OD_{Sample}-OD_{Control})}{(OD_{Standard} - OD_{Blank}) \times V_{Sample} \times C_{Protein} \times T} \ (U/mg)$$

2. 2According to the quantity of cells or bacteria

$$\text{Activity} = \frac{(C_{Standard} \times V_{Standard}) \times (OD_{Sample}-OD_{Control})}{(OD_{Standard} - OD_{Blank}) \times N \times (V_{Sample} / V_{Assay}) \times T} \ (U/10^4)$$

2. 3 According to the weight of sample

$$\text{Activity} = \frac{(C_{Standard} \times V_{Standard}) \times (OD_{Sample}-OD_{Control})}{(OD_{Standard} - OD_{Blank}) \times W \times (V_{Sample} / V_{Assay}) \times T} \ (U/g)$$

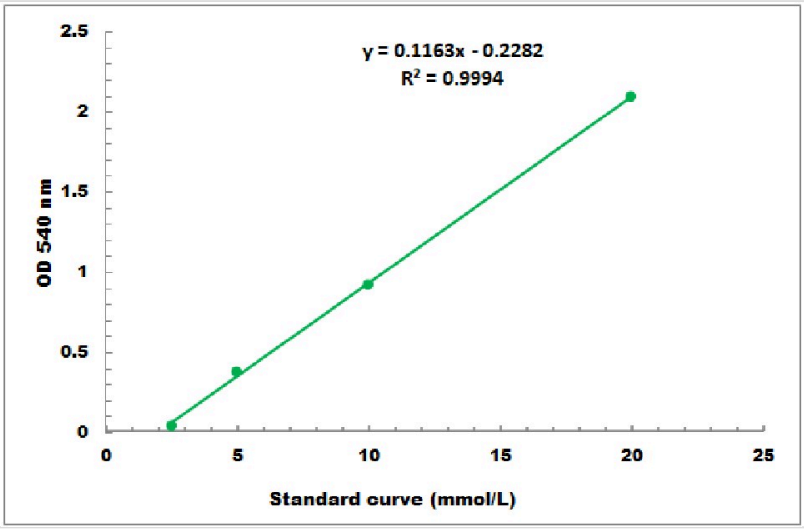
2. 4 According to the volume of sample

$$\text{Activity} = \frac{(C_{Standard} \times V_{Standard}) \times (OD_{Sample}-OD_{Control})}{(OD_{Standard} - OD_{Blank}) \times V_{Sample} \times T} \ (U/mL)$$

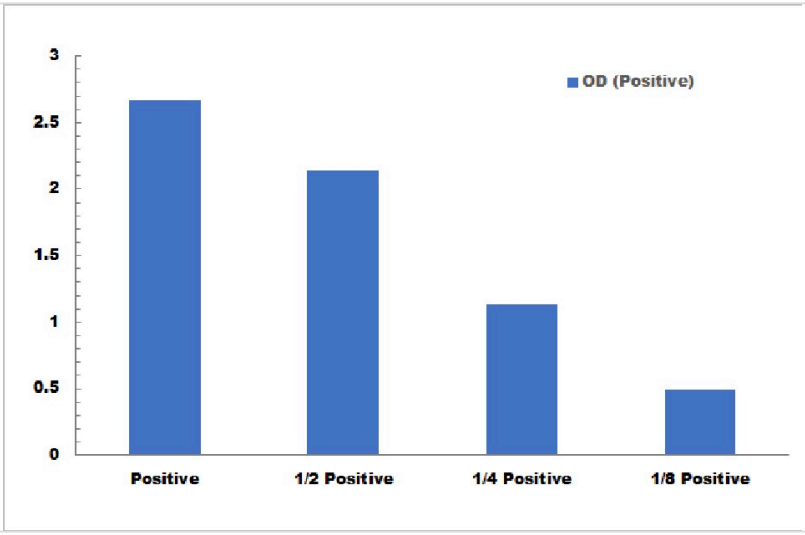
Item	Definition
Slope	the absorbance slope of standard curve
n	the dilution factor
$C_{Protein}$	the protein concentration of sample, mg/mL
W	the weight of total sample , g
N	the quantity of total cell or bacteria sample, 10^4
$C_{Standard}$	the concentration of standard, mmol/L = μmol/mL
$V_{Standard}$	the volume of standard in assay procedure, mL
V_{Sample}	the volume of sample in assay procedure, mL
V_{Assay}	the volume of Assay Buffer in sample preparation , mL
T	the reaction time , minute

8. Typical Data

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



Detection Range : 2mmol/ L -20mmol/ L



Positive Control reaction in 96 -well plate assay with decreasing the concentration