

# Sucrase Microplate Assay Kit

## Basic information:

Catalog No.: UAK1037

Size: 100 Assays

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

## I. INTRODUCTION

Sucrase is the name given to a number of enzymes located in on the brush border of the small intestine that catalyze the hydrolysis of sucrose to fructose and glucose. The enzyme invertase, which occurs more commonly in plants, also hydrolyzes sucrose but by a different mechanism.

The enzyme catalysed reaction products can be measured at a colorimetric readout at 540 nm.

## II. KIT COMPONENTS

Component	Volume	Storage
96-Well Microplate	1 plate	
Assay Buffer	30 ml x 4	4 °C
Substrate	4 ml x 1	4 °C
Stop Solution	2 ml x 1	4 °C
Dye Reagent	10 ml x 1	4 °C
Standard	Powder x 1	4 °C
Plate Adhesive Strips	3 Strips	
Technical Manual	1 Manual	

### Note:

**Standard:** add 1 ml distilled water to dissolve before use, the concentration will be 5 mmol/L.

## III. MATERIALS REQUIRED BUT NOT PROVIDED

1. Microplate reader to read absorbance at 540 nm
2. Distilled water
3. Pipettor
4. Pipette tips
5. Mortar
6. Centrifuge
7. Timer
8. Ice
9. Convection oven

#### **IV. 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  $5 \times 10^6$  cell or bacteria, sonicate (with power 20%, sonication 3s, interval 10s, repeat 30 times); centrifuged at 8000g 4 °C for 10 minutes, 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 8000g 4 °C for 10 minutes, take the supernatant into a new centrifuge tube and keep it on ice for detection.

3. For liquid samples

Detect directly.

#### **V. ASSAY PROCEDURE**

Add following reagents into the microplate:

Reagent	Sample	Control	Standard	Blank
Sample	40 $\mu$ l	--	--	--
Stop Solution	--	20 $\mu$ l	--	--

Substrate	40 µl	40 µl	--	--
Mix, put it in the oven, 37 °C for 5 minutes.				
Stop Solution	20 µl	--	--	--
Standard	--	--	40 µl	--
Distilled water	--	40 µl	60 µl	100 µl
Dye Reagent	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.				

## VI. CALCULATION

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

1. According to the protein concentration of sample

$$\begin{aligned} \text{Sucrase (U/mg)} &= C_{\text{Standard}} \times V_{\text{Standard}} \times (\text{OD}_{\text{Sample}} - \text{OD}_{\text{Control}}) / (\text{OD}_{\text{Standard}} - \text{OD}_{\text{Blank}}) / \\ &\quad (V_{\text{Sample}} \times C_{\text{Protein}}) / T \\ &= (\text{OD}_{\text{Sample}} - \text{OD}_{\text{Control}}) / (\text{OD}_{\text{Standard}} - \text{OD}_{\text{Blank}}) / C_{\text{Protein}} \end{aligned}$$

2. According to the weight of sample

$$\begin{aligned} \text{Sucrase (U/g)} &= C_{\text{Standard}} \times V_{\text{Standard}} \times (\text{OD}_{\text{Sample}} - \text{OD}_{\text{Control}}) / (\text{OD}_{\text{Standard}} - \text{OD}_{\text{Blank}}) / (W \times \\ &\quad V_{\text{Sample}} / V_{\text{Assay}}) / T \\ &= (\text{OD}_{\text{Sample}} - \text{OD}_{\text{Control}}) / (\text{OD}_{\text{Standard}} - \text{OD}_{\text{Blank}}) / W \end{aligned}$$

3. According to the quantity of cell or bacteria

$$\begin{aligned} \text{Sucrase (U/10}^4\text{)} &= C_{\text{Standard}} \times V_{\text{Standard}} \times (\text{OD}_{\text{Sample}} - \text{OD}_{\text{Control}}) / (\text{OD}_{\text{Standard}} - \text{OD}_{\text{Blank}}) / (N \times \\ &\quad V_{\text{Sample}} / V_{\text{Assay}}) / T \\ &= (\text{OD}_{\text{Sample}} - \text{OD}_{\text{Control}}) / (\text{OD}_{\text{Standard}} - \text{OD}_{\text{Blank}}) / N \end{aligned}$$

4. According to the volume of sample

$$\begin{aligned} \text{Sucrase (U/ml)} &= C_{\text{Standard}} \times V_{\text{Standard}} \times (\text{OD}_{\text{Sample}} - \text{OD}_{\text{Control}}) / (\text{OD}_{\text{Standard}} - \text{OD}_{\text{Blank}}) / \\ &\quad V_{\text{Sample}} / T \\ &= (\text{OD}_{\text{Sample}} - \text{OD}_{\text{Control}}) / (\text{OD}_{\text{Standard}} - \text{OD}_{\text{Blank}}) \end{aligned}$$

$C_{\text{Protein}}$ : the protein concentration, mg/ml;

$C_{\text{Standard}}$ : the concentration of Standard, 5 mmol/L = 5 µmol/ml;

W: the weight of sample, g;

N: the quantity of cell or bacteria,  $N \times 10^4$ ;

$V_{\text{Sample}}$ : the volume of sample, 0.04 ml;

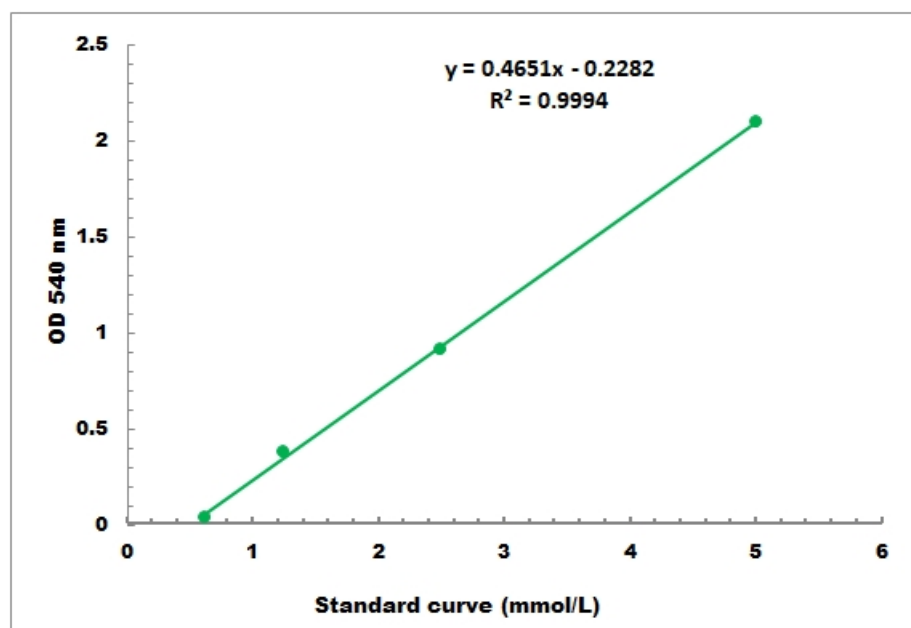
$V_{\text{Standard}}$ : the volume of sample, 0.04 ml;

$V_{\text{Assay}}$ : the volume of Assay buffer, 1 ml;

T: the reaction time, 5 minutes.

## VII. TYPICAL DATA

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



Detection Range: 0.5 mmol/L - 5 mmol/L