Acid Invertase Microplate Assay Kit

Catalog # AS0026

Detection and Quantification of Acid Invertase Activity in Tissue extracts, Cell lysate Samples.

This instruction must be read in its entirety before using this product.

For research use only, Not for use in diagnostic procedures.

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I. INTRODUCTION

Invertase, also known as sucrase or β -fructofuranosidase, catalyzes the hydrolysis of sucroseby cleaving its glycosidic bond and forming one molecule each of glucose and fructose.According to the optimum pH,invertase is divided into acid invertase (AI) and neutral invertase (NI).

AI mainly exists in cell vacuole or free space, the optimum pH is 4.5 ~5.0.AI regulates the use of sugar in vacuole and the accumulation of sugar in fruit through the degradation of sucrose in the vacuole.

AI hydrolyzes the sucrose to generate reducing sugar. The reducing sugar reduces the 3,5-dinitrosalicylic acid to generate red-brown substance.The color intensity, measured at 540 nm, is proportionate to the enzyme activity in the sample.

II.KIT COMPONENTS

Component	Volume	Storage
96-Well Microplate	1 plate	
Assay Buffer	30 mlx 4	4 °C
Reaction Buffer	20 ml x 1	4 °C
Substrate	Powderx 1	4 °C
Dye Reagent	10 mlx 1	4 °C
Standard	Powderx 1	4 °C
Plate Adhesive Strips	3 Strips	
Technical Manual	1 Manual	

Note:

Substrate: add 8 ml Reaction Buffer to dissolve before use.

Standard:add 1 mldistilled water to dissolve before use, the concentration will be 3 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. Ice
7. Centrifuge
8. Timer
9.Convection oven

IV. SAMPLE PREPARATION

1.For tissue samples

Weighout 0.1 g tissue, homogenize with 1 mlAssay buffer on ice, centrifuged at 12000g 4°C for 10minutes, take the supernatant into a new centrifuge tube and keep it on ice for detection.

2.For cell and bacteria samples

Collect cell or bacteria into centrifuge tube, discard the supernatant after centrifugation, add 1 mlAssay buffer for 5 $\times 10^6$ cell or bacteria, sonicate (with power 20%, sonication 3s, intervation 10s,repeat 30 times); centrifuged at 12000g 4°C for 10minutes, take the supernatant into a new centrifuge tube and keep it on ice for detection.

V. ASSAY PROCEDURE

Add following reagents in the microcentrifuge tubes:

Reagent	Sample	Control	Standard	Blank
Sample	20 µl	--	--	--
Reaction Buffer	--	20 µl	--	--
Substrate	80 µl	80 µl	--	--
Mix, put it in the oven,37°C for 30minutes. Then put it in boiling waterfor 10 minutes. Add the supernatant into the microplate.				
Supernatant	100 µl	100 µl	--	--
Standard	--	--	100 µl	--
Distilled water	--	--	--	100 µl
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.				

VI. CALCULATION

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

1. According to the protein concentration of sample

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

$= 0.5 \times (OD_{Sample} - OD_{Control}) / (OD_{Standard} - OD_{Blank}) / C_{Protein}$

2. According to the weight of sample

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

$= 0.5 \times (OD_{Sample} - OD_{Control}) / (OD_{Standard} - OD_{Blank}) / W$

3. According to the quantity of cell or bacteria

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

$= 0.5 \times (OD_{Sample} - OD_{Control}) / (OD_{Standard} - OD_{Blank}) / N$

$C_{Standard}$: the concentration of Standard, 3mmol/L = 3μmol/ml;

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

W: the weight of sample, g;

$V_{Standard}$: the volume of standard, 0.1 ml;

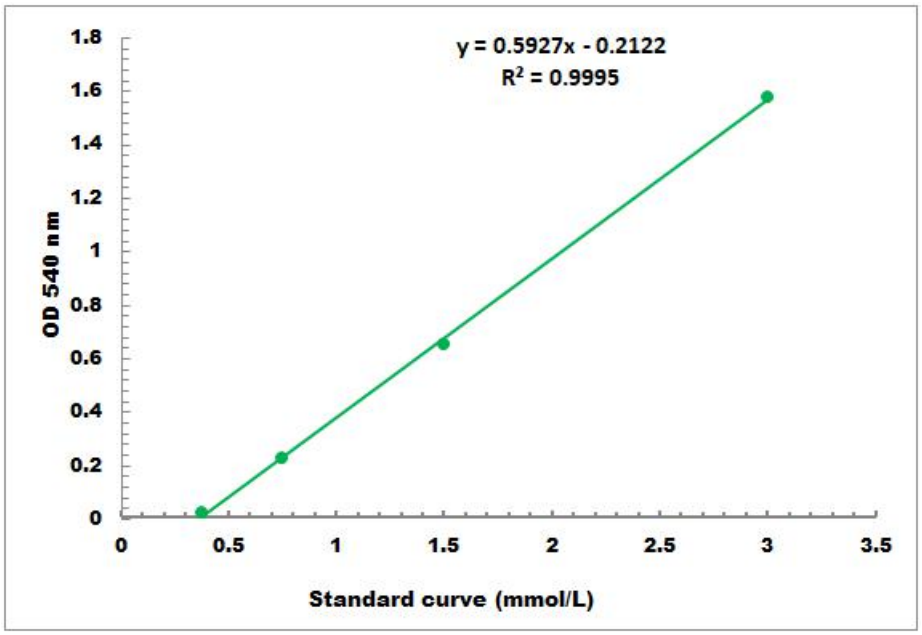
V_{Sample}: the volume of sample, 0.02 ml;

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

T: the reaction time, 30 minutes.

VII. TYPICAL DATA

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



Detection Range: 0.3mmol/L -3mmol/L

VIII. TECHNICAL SUPPORT

For troubleshooting, information or assistance, please go online to www.sabbiotech.cn or contact us at techcn@signalwayantibody.com

IX. NOTES