



Acid Invertase Microplate Assay Kit

User Manual

Catalog # CAK1026

(Version 1.2D)

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

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

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I. INTRODUCTION

Invertase, also known as sucrase or β -fructofuranosidase, catalyzes the hydrolysis of sucrose by 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 ml x 4	4 °C
Reaction Buffer	20 ml x 1	4 °C
Substrate	Powder x 1	4 °C
Dye Reagent	10 ml x 1	4 °C
Standard	Powder x 1	4 °C
Positive Control	Powder x 1	-20 °C
Plate Adhesive Strips	3 Strips	
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Note:

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

Standard: add 1 ml distilled water to dissolve before use, mix; then add 0.3 ml into 0.7 ml distilled water, the concentration will be 3 mmol/L.

Positive Control: add 1 ml Assay Buffer to dissolve before use, then add 0.1 ml into Assay Buffer 0.9 ml.

III. 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. Ice
7. Centrifuge
8. Timer
9. Convection oven

IV. SAMPLE PREPARATION

1. For tissue samples

Weigh out 0.1 g tissue, homogenize with 1 ml Assay buffer on ice, centrifuged at 12000g 4 °C for 10 minutes, 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 ml Assay buffer for 5×10^6 cell or bacteria, sonicate (with power 20%, sonicate 3s, interval 10s, repeat 30 times); centrifuged at 12000g 4 °C for 10 minutes, 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	Positive Control
Sample	20 μ l	--	--	--	--
Reaction Buffer	--	20 μ l	--	--	--
Positive Control	--	--	--	--	20 μ l
Substrate	80 μ l	80 μ l	--	--	80 μ l
Mix, put it in the oven, 37 °C for 10 minutes.					
Standard	--	--	100 μ l	--	--
Distilled water	--	--	--	100 μ l	--
Dye Reagent	100 μ l	100 μ l	100 μ l	100 μ l	100 μ l
Mix, put it into the convection oven, 90 °C for 10 minutes, record absorbance measured at 540nm.					

Note:

- 1) Perform 2-fold serial dilutions of the top standards to make the standard curve.
- 2) 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 sample into the reaction system; or increase the reaction time; if the enzyme activity is higher, please dilute the sample, or decrease the reaction time.
- 3) Reagents must be added step by step, can not be mixed and added together.

VI. CALCULATION

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

1. According to the protein concentration of sample

$$\begin{aligned} \text{AI (U/mg)} &= C_{\text{Standard}} \times V_{\text{Standard}} \times (\text{OD}_{\text{Sample}} - \text{OD}_{\text{Control}}) / (\text{OD}_{\text{Standard}} - \text{OD}_{\text{Blank}}) / (V_{\text{Sample}} \times \\ & C_{\text{Protein}}) / T \\ &= 1.5 \times (\text{OD}_{\text{Sample}} - \text{OD}_{\text{Control}}) / (\text{OD}_{\text{Standard}} - \text{OD}_{\text{Blank}}) / C_{\text{Protein}} \end{aligned}$$

2. According to the weight of sample

$$\begin{aligned} \text{AI (U/g)} &= C_{\text{Standard}} \times V_{\text{Standard}} \times (\text{OD}_{\text{Sample}} - \text{OD}_{\text{Control}}) / (\text{OD}_{\text{Standard}} - \text{OD}_{\text{Blank}}) / (W \times V_{\text{Sample}} \\ & / V_{\text{Assay}}) / T \\ &= 1.5 \times (\text{OD}_{\text{Sample}} - \text{OD}_{\text{Control}}) / (\text{OD}_{\text{Standard}} - \text{OD}_{\text{Blank}}) / W \end{aligned}$$

3. According to the quantity of cell or bacteria

$$\begin{aligned} \text{AI (U/10}^4) &= C_{\text{Standard}} \times V_{\text{Standard}} \times (\text{OD}_{\text{Sample}} - \text{OD}_{\text{Control}}) / (\text{OD}_{\text{Standard}} - \text{OD}_{\text{Blank}}) / (N \times \\ & V_{\text{Sample}} / V_{\text{Assay}}) / T \\ &= 1.5 \times (\text{OD}_{\text{Sample}} - \text{OD}_{\text{Control}}) / (\text{OD}_{\text{Standard}} - \text{OD}_{\text{Blank}}) / N \end{aligned}$$

C_{Standard} : the concentration of Standard, 3 mmol/L = 3 $\mu\text{mol/ml}$;

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

W: the weight of sample, g;

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

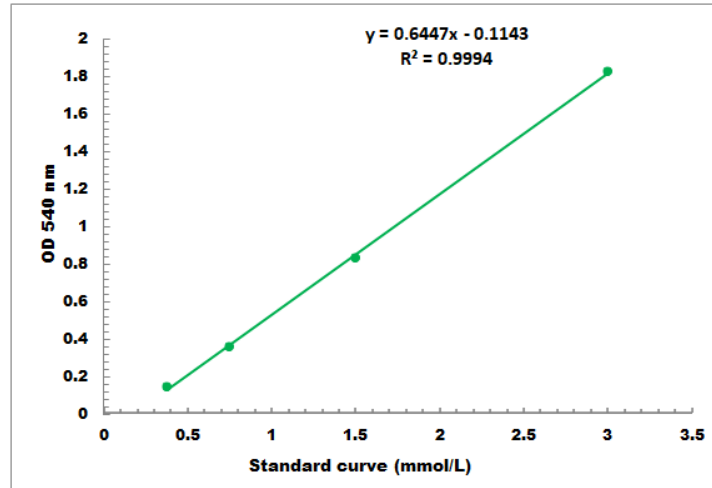
V_{Sample} : the volume of sample, 0.02 ml;

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

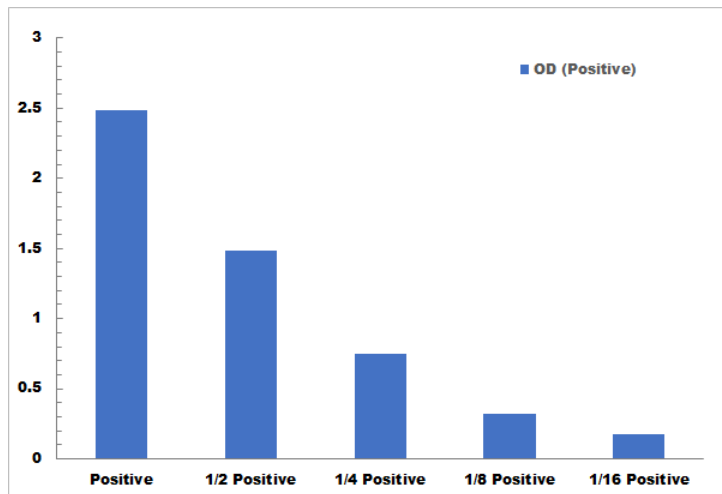
T: the reaction time, 10 minutes.

VII. TYPICAL DATA

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



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



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

VIII. TECHNICAL SUPPORT

For troubleshooting, information or assistance, please go online to www.cohesionbio.com or contact us at techsupport@cohesionbio.com

IX. NOTES