Acid Invertase(AI) Activity Assay Kit

Product Code: 112897

Acid Invertase (AI) Activity Assay Kit - Microplate Method

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Pack size: 100T

Product Overview

Invertase (Ivr) catalyzes the irreversible decomposition of sucrose into fructose and glucose and is one of the key enzymes in sucrose metabolism in higher plants. According to optimum pH, Ivr is classified as acid invertase (AI) and neutral invertase (NI).

AI has an optimum pH of 3-5. AI is divided into soluble AI (S-AI) and cell wall-insoluble AI (B-AI). S-AI mainly exists in cell vacuoles or the free space, with an optimum pH of 4.5-5.0. By degrading sucrose in vacuoles, it regulates sucrose utilization in vacuoles and the accumulation of sugars in fruits.

S-AI catalyzes the degradation of sucrose to produce reducing sugars. These reducing sugars further react with 3,5-dinitrosalicylic acid to form a brownish-red amino compound with characteristic light absorption at 540 nm. Within a certain range, the increase in light absorption at 540 nm is proportional to S-AI activity.

Package Contents

Item Code	Item	Quantity
112897.1	Reagent I	1 bottle
112897.2	Reagent II	1 bottle
112897.3	Reagent III	1 bottle
112897.4	Extraction Solution	1 bottle
112897.m	Manual	1 copy

Quality Standards and Safety

Raw Material and Packaging Name	Quality Standards	Primary Toxicity
Reagent I	-	-
Reagent II	-	-
Reagent III	-	-
Extraction Solution	-	-

Transport and Storage

Transportation: Transport this product with ice packs.

Storage: Store at 2-8 °C, protected from light. Shelf life: 180 days.

Instructions for Use

1. Crude Enzyme Extract Preparation

1. Prepare the extract according to a tissue mass (g) to extraction solution volume (mL) ratio of 1:5-10.
2. Recommended preparation: weigh about 0.1 g tissue and add 1 mL extraction solution.
3. Homogenize in an ice bath.

4. Centrifuge at 12000 g and 4 °C for 10 min.
5. Collect the supernatant and keep it on ice for testing.

2. Reagent Preparation

1. Before use, add 10 mL of Reagent I to Reagent II.
2. Dissolve completely and set aside.
3. Store any unused reagent at 4 °C.

3. Assay Procedure

Preheat the microplate reader for 30 min or longer, adjust the wavelength to 540 nm, and zero with distilled water.

Component	Test Tube	Control Tube
Sample (μL)	50	50
Reagent I (μL)	200	-
Reagent II (μL)	-	200

1. Mix well.
2. Incubate in a 37 °C water bath for exactly 30 min.
3. Then place in a 95 °C water bath for 10 min. Cap tightly to prevent water loss.
4. Cool under running water and mix thoroughly to ensure the concentration remains unchanged.
5. Centrifuge at 12000 g and 4 °C for 5 min.
6. Collect the supernatant.

Component	Test Tube	Control Tube
Supernatant (μL)	200	200
Reagent III (μL)	125	125

1. Mix well.
2. Incubate in a 95 °C water bath for 10 min. Cap tightly to prevent water loss.
3. Cool under running water and mix thoroughly.
4. Transfer 200 μL to a 96-well plate.
5. Record the absorbance value of each tube (A) at 510 nm.
6. If the absorbance value is greater than 2, dilute with distilled water and remeasure. Multiply by the corresponding dilution factor in the calculation formula.
7. $\Delta A = A_{determination} - A_{control}$.
8. Each determination tube requires one control tube.

Activity Calculation

Regression equation determined under standard conditions: y = 0.0008x - 0.001, where x is the standard concentration (μg/mL) and y is the absorbance.

1. Calculated by Protein Concentration

Unit definition: at 37 °C, the production of 1 μg reducing sugar per minute per mg protein is defined as one enzyme activity unit.

S-AI Activity (μg/min/mg prot) = $[((\Delta A + 0.001) / 0.0008) \times V_1] / (V_1 \times Cpr) / T = 41.6 \times (\Delta A + 0.001) / Cpr$

2. Calculated by Fresh Weight

Unit definition: at 37 °C, the production of 1 μg reducing sugar per minute per g tissue is defined as one enzyme activity unit.

S-AI Activity (μg/min/g fresh weight) = $[((\Delta A + 0.001) / 0.0008) \times V_1] / (W \times V_1 / V_2) = 41.6 \times (\Delta A + 0.001) / W$

- V_1: sample volume added to the reaction system, 0.05 mL
- V_2: volume of added extraction solution, 1 mL
- T: reaction time, 30 min
- Cpr: sample protein concentration, mg/mL
- W: sample fresh weight, g

Notes

> Before formal determination, select 2-3 samples with large expected differences for pre-testing.
>
> Required instruments and supplies: microplate reader, benchtop centrifuge, water bath, pipette, 96-well plate, mortar, ice, and distilled water.

Visual Reference