Acid Invertase(AI) Activity Assay Kit

Product Code: 112896

112896 Acid Invertase (AI) Activity Assay Kit - Micro Method

Product Introduction

Sucrose invertase (Invertase, Ivr) catalyzes the irreversible breakdown of sucrose into fructose and glucose and is one of the key enzymes in sucrose metabolism in higher plants. According to optimum pH, invertase is divided into acid invertase (AI) and neutral invertase (NI). AI has an optimum pH of 3-5.

AI includes soluble acid invertase (S-AI) and cell wall-insoluble acid invertase (B-AI). S-AI is mainly present 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 sugar accumulation in fruits.

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

Package Contents

Pack Size	Code	Item	Quantity
50T	112896.1	Reagent I	1 bottle
50T	112896.2	Reagent II	1 bottle
50T	112896.3	Reagent III	1 bottle
50T	112896.4	Extraction Solution	1 bottle
50T	112896.m	Manual	1 copy

Quality Standards and Safety Information

Raw Material and Packaging Name	Quality Standard	Main Toxicity
Reagent I	---	---
Reagent II	---	---
Reagent III	---	---
Extraction Solution	---	---

Transportation and Storage

Transport with ice packs.

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

Instructions for Use

1. Crude Enzyme Extraction

1. Use a tissue mass (g) to extraction solution volume (mL) ratio of 1:5-10.
2. The recommended preparation is about 0.1 g tissue with 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

Before use, add Reagent II to 10 mL of Reagent I and dissolve completely. Unused reagent can be stored at 4°C.

3. Assay Procedure

The assay uses sample tubes and matching control tubes. Each assay tube requires a control tube.

3.1 Instrument Preparation

1. Preheat the spectrophotometer or microplate reader for 30 min or longer.
2. Set the wavelength to 540 nm.
3. Zero the instrument with distilled water.

3.2 Reaction Setup

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

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

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

1. Mix well and incubate in a 95°C water bath for 10 min. Cap tightly to prevent moisture loss.
2. Cool under running water and mix thoroughly.
3. Transfer 200 μL to a micro quartz cuvette or a 96-well plate.
4. Record the absorbance value of each tube as A at 510 nm.
5. If the absorbance is greater than 2, dilute with distilled water before measurement and multiply by the corresponding dilution factor in the calculation formula.
6. Calculate ΔA = Ameasured - Acontrol.

Note: The procedure text specifies 540 nm for instrument setup and 510 nm for absorbance reading. Keep the original stated values when following the source procedure.

Activity Calculation

4.1 Micro Quartz Cuvette Method

Regression equation under standard conditions: y = 0.0016x - 0.001, where x is the standard concentration (μg/mL) and y is the absorbance.

Calculated by Protein Concentration

Unit definition: At 37°C, the amount of enzyme in each mg of protein that produces 1 μg reducing sugar per minute is defined as one enzyme activity unit.

S-AI activity (μg/min/mg prot) = [((ΔA + 0.001) ÷ 0.0016 × V1)] ÷ (V1 × Cpr) ÷ T = 20.8 × (ΔA + 0.001) ÷ Cpr

Calculated by Fresh Weight

Unit definition: At 37°C, the amount of enzyme in each g of tissue that produces 1 μg reducing sugar per minute is defined as one

enzyme activity unit.

S-AI activity (μg/min/g fresh weight) = [((ΔA + 0.001) ÷ 0.0016 × V1)] ÷ (W × V1 ÷ V2) ÷ T = 20.8 × (ΔA + 0.001) ÷ W

4.2 96-Well Plate Method

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

Calculated by Protein Concentration

Unit definition: At 37°C, the amount of enzyme in each mg of protein that produces 1 μg reducing sugar per minute is defined as one enzyme activity unit.

S-AI activity (μg/min/mg prot) = [((ΔA + 0.001) ÷ 0.0008 × V1)] ÷ (V1 × Cpr) ÷ T = 41.6 × (ΔA + 0.001) ÷ Cpr

Calculated by Fresh Weight

Unit definition: At 37°C, the amount of enzyme in each g of tissue that produces 1 μg reducing sugar per minute is defined as one enzyme activity unit.

S-AI activity (μg/min/g fresh weight) = [((ΔA + 0.001) ÷ 0.0008 × V1)] ÷ (W × V1 ÷ V2) = 41.6 × (ΔA + 0.001) ÷ W

Symbol	Definition
V1	Sample volume added to the reaction system, 0.05 mL
V2	Volume of extraction solution added, 1 mL
T	Reaction time, 30 min
Cpr	Sample protein concentration, mg/mL
W	Sample fresh weight, g

Notes

- Before formal measurement, select 2-3 samples with large expected differences for prediction.
- Required instruments and supplies: visible spectrophotometer or microplate reader, benchtop centrifuge, water bath, pipette, cuvette or 96-well plate, mortar, ice, and distilled water.

Visual Reference