# Acid Invertase (AI) Activity Assay Kit

**Note:** Take two or three different samples for prediction before test.

**Operation Equipment:** Spectrophotometer

**Catalog Number:** NA0382

**Size:** 100T/48S

**Components:**

Extract solution:50 mL×1. Storage at 4°C.

Reagent I: 60 mL×1. Storage at 4°C.

Reagent II: Powder×1. Storage at 4°C. Add 30 mL of Reagent I to fully dissolve for standby when the solution will be used. Unused reagent is still stored at 4°C.

Reagent III: 35 mL×1. Storage at 4°C.

Standard solution: powder×1, 10 mg of glucose. Storage at 4°C. Add 1 mL of distilled water with filly dissolve before use to prepare 10 mg/mL glucose standard solution for standby.

**Product Description**

Invertase (Ivr) catalyzes the irreversible decomposition of sucrose into fructose and glucose, which is one of the key enzymes in sucrose metabolism of higher plants. According to the optimal pH, Ivr can be divided into two types: acid invertase (AI) and neutral invertase (Ni).

AI (EC 3.2.1.26) mainly exists in cell vacuole or free space, and the optimal pH is 4.5-5.0 (acid). It can regulate the utilization of sucrose in vacuole and the accumulation of sugar in fruit by degrading sucrose in vacuole.

AI catalyzes the degradation of sucrose to produce reducing sugar, and further reacts with 3,5-dinitrosalicylic acid to form brownish red amino compound, which has a characteristic light absorption at 540 nm. the increase rate of light absorption at 540 nm in a certain range is in direct proportion to AI activity.

**Reagents and Equipment Required but Not Provided**

Spectrophotometer, desktop centrifuge, water-bath, adjustable pipette, 1 mL glass cuvette, mortar/homogenizer, ice and distilled water.

**Procedure**

**I. Extraction of crude enzyme**

Weigh about 0.1 g of tissue, add 1 mL of Extract solution for ice bath homogenization. Centrifuge at 12000×g for 10 minutes at 4°C, take the supernatant and place it on ice for test.

**II. Determination steps and sample adding table:**

1. Preheat spectrophotometer more than 30 minutes, adjust wavelength to 540 nm and set zero with distilled water.

2. Dilute the standard solution to 1.0, 0.8, 0.6, 0.4, 0.2 and 0 mg/mL of glucose standard solution.
3. Sample determination (add the following reagents in sequence in the 1.5 mL EP tube):

| Reagent Name (μL) | Test tube (T) | Control tube (C) | Standard tube (S) |
|---|---|---|---|
| Crude enzyme | 200 | 200 | - |
| Reagent I | - | 800 | - |
| Reagent II | 800 | - | 800 |
| Standard solution | - | - | 200 |
| Mix well. After 30 minutes of accurate water bath at 37°C, boil for about 10 minutes (cover tightly to prevent water loss). After water cooling, mix well (to ensure constant concentration). Centrifuge at 12000 ×g for 5 minutes at 4°C and take the supernatant. | | | |
| Supernatant | 900 | 900 | 900 |
| Reagent III | 500 | 500 | 500 |

Mix well, boil for about 10 minutes (cover tightly to prevent water loss). After water cooling, mix well. record the absorption value A of each tube at 540 nm, calculate $\Delta A = A_T - A_C$.

**Calculation of AI activity:**

1. Production of standard curve：Draw the standard curve with the absorbance value of each concentration minus the absorbance of the blank tube (concentration of 0 mg/mL) as the y-axis and the glucose concentration as the x-axis. Take ΔA into the equation to get x (μmol/mL).
2. Calculation of AI activity:
1) Calculate by protein concentration:
Unit definition: One unit of enzyme activity is defined as the amount of enzyme catalyzes the production of 1 μg of reducing sugar per minute at 37°C every milligram of protein.
AI activity(U/mg) = (x×V1×1000)÷(V1×Cpr)÷T=33.3×x÷Cpr
2) Calculate by sample fresh weight:
Unit definition: One unit of enzyme activity is defined as the amount of enzyme catalyzes the production of 1 μg of reducing sugar per minute at 37°C every gram of tissue.
AI activity (U/g fresh weight) = (x×V1×1000)÷(W×V1÷V2)÷T=33.3×x÷W

1000: Unit conversion factor, 1 mg/mL = 1000 μg/mL;
V1: The volume of sample added into the reaction system, 0.2 mL;
V2: Add the volume of extract solution, 1 mL;
Cpr: Concentration of sample protein, mg/mL;
W: Sample fresh weight, g;
T: Reaction time: 30 minutes.

**Note**

1. If Reagent III is added and there is turbidity after boiling for 10 minutes, it is recommended to remove the precipitate by centrifugation and take the supernatant to determine the absorbance.
2. If the absorbance value is greater than 1, the sample can be measured after diluted with distilled water

(multiply the corresponding dilution times in the calculation formula).
3. Because the Extract solution contains a certain concentration of protein (about 1mg/mL), the protein content of the Extract solution itself needs to be subtracted when determining the protein concentration of the sample.

**Experimental example:**

1. Take 0.1g of the heart of yellow flower and add 1 mL of Extract solution for homogenization and grinding, take the supernatant and dilute it twice with distilled water, and then operate according to the determination steps. $\Delta A_T$ = 0.918, $\Delta A_C$ = 0.752, $\Delta A = A_T - A_C$ = 0.918-0.752 = 0.166, bring in the standard curve y = 1.3885x - 0.1929, calculate x = (0.166 + 0.1929)/1.3885 = 0.25848
AI activity (U / g mass) = 33.3 × x ÷ W × dilution ratio = 33.3×0.25848÷0.1=86.074 U/g mass.

**References:**

[1] Huang Y W, Nie Y X, Wan Y Y, et al. Exogenous glucose regulates activities of antioxidant enzyme, soluble acid invertase and neutral invertase and alleviates dehydration stress of cucumber seedlings[J]. Scientia horticulturae, 2013, 162: 20-30.

**Related Products:**

NA0582/NA0824 Neutral Invertase(NI) Activity Assay Kit
NA0823/NA0581 Sucrose Synthetase(SS) Activity Assay Kit
NA0821/NA0579 Sucrose Phosphoric Acid Synthetase(SPS) Activity Assay Kit
NA0694/NA0453 Plant Sucrose Content Assay Kit