SunLong Biotech Co.,LTD
Tel: 0086-571-56623320 Fax:0086-571-56623318
E-mail:sales@sunlongbiotech.com
www.sunlongbiotech.com

Cell Wall-Bound Acid Invertase (CWI) Activity Assay Kit

Note: It is necessary to predict 2-3 large difference samples before the formal determination. **Operation Equipment:** Spectrophotometer/ microplate reader

Cat No: AK0081

Size: 100T/48S

Components:

Extract solution I: Liquid 60mL×1, store at 4℃;

Extract solution II: Liquid 100mL×1, store at 4℃;

Reagent I: Liquid 45mL×1, store at 4℃;

Reagent II: Powder×1, store at 4℃ . Add 20mL of Reagent I when the solution will be used. The rest of reagent store at 4℃;

Reagent III: Liquid 25 mL×1, store at 4℃;

Standard: Powder×1, 10 mg glucose. before use, add 1 mL of distilled water to dissolve to prepare 10 mg/mL glucose standard solution. Storage at 4℃ for one week.

Product Description:

Sucrose invertase (Invertase, Inv) irreversibly catalyzes sucrose to form glucose and fructose, which plays an important role in plant sucrose metabolism. According to the optimum pH, Inv can be divided into acid invertase (AI) and neutral invertase (NI). Among them, AI can be divided into soluble acid invertase (SAI) and cell wall-bound acid Invertase (CWI) according to the difference of subcellular localization. CWI is bound to the cell wall in the form of ionic bonds, and plays an important role in sucrose unloading, sucrose extracellular transport, plant growth and development, and resistance to various stresses in the phloem.

CWI catalyzes the degradation of sucrose to produce reducing sugar. The reducing sugar reacts with 3,5-dinitrosalicylic acid to produce a brown-red substance with a characteristic absorption peak at 540 nm. The CWI activity can be calculated by measuring the change in absorbance at 540 nm.

Required but Not Provided:

Spectrophotometer/microplate reader, desk centrifuge, water-bath, transferpettor, mortar/homogenizer, micro glass cuvette/ 96-well flat-bottom plate, ice, EP tube and distilled water.

Protocol

I. Preparation:

Tissue: According to the quality (g): extract solution I (mL)=1: 5~10 (recommend to weigh about 0.1 g, add 1 mL of extract solution I) to add the extract solution I, homogenize on an ice bath, centrifuge at 12000g and 4℃ for 10 min, discard the supernatant and leave the pellet. Add 1 mL of distilled water to the pellet and mix thoroughly with shaking, centrifuge at 12000 g and 4℃ for 10 min, discard the supernatant and leave the pellet; add 1 mL of extract solution II to the pellet and thoroughly mix, extract for 15 h at

4℃ , then centrifugated at 12000 g and 4℃ for 10 min,, and take the supernatant on ice for test.

II. Determination procedure:

1. Preheat spectrophotometer/ microplate reader for 30 minutes, adjust wavelength to 540 nm, set the counter to zero with distilled water.

2. Dilute 10 mg/mL standard solution with distilled water to 2, 1, 0.8, 0.6, 0.5, 0.4, 0.3 mg/mL standard solution for use.

3. Operation table:

Reagent name （μL）	Control tube (Ac)	Test tube (At)	Standard tube (As)	Blank tube (Ab)
sample	80	80	-	-
Standard	-	-	80	-
Distilled water	-	-	-	80
Reagent I	320	-	320	320
Reagent II	-	320	-	-
Mix well, react accurately for 30min in 37℃ water bath, then put the react solution in 95℃ water bath for 10 min (close tightly to prevent water loss).				
Reagent III	200	200	200	200
Mix well and boil in a boiling water bath for 5 minutes (close tightly to prevent water loss), and immediately cool to room temperature after taking out. Take 200μL into micro glass cuvette/ 96-well plate. The absorbance A at 540 nm is measured and recorded as Ac, At, As, and Ab. Calculate ΔA = At-Ac, ΔAs = As-Ab. A control tube is required for each measurement tube, and the standard curve need only be detected 1-2 times.				

Note: If precipitation occurs during the first 95℃ water bath step, it is recommended to centrifuge at 12,000 g at room temperature for 5 minutes and take the supernatant (if the supernatant is less than 400 μL, the sample system can be scaled down to the next step, such as300μL supernatant + 150 μL Reagent III).

CWI Calculation:

1. According to concentration of standard solution and absorbance to create the standard curve, take standard solution as X-axis, ΔAs as Y-axis. Take ΔA into the equation to obtain x (nmol/mL).

2. Protein concentration:

Unit definition: One unit of enzyme activity is defined as the amount of enzyme that catalyzes the generation of 1 μg of reducing sugar per minutes in the reaction system every milligram tissue protein.

CWI (U/mg prot) = x× Ve ÷ (Ve ×Cpr)×1000÷T =33.33x÷Cpr

3. Sample weight:

Unit definition: One unit of enzyme activity is defined as the amount of enzyme that catalyzes the generation of 1 μg of reducing sugar per minutes in the reaction system every gram tissue

CWI (U/g) = x× Ve÷ W×1000÷T =33.33x÷W

Ve: volume used in the extraction solution, 1mL;

Cpr: sample protein concentration, mg/mL;

W: Fresh weight of sample, g;

T: React time, 30min.

Note:

1. When A or ΔA exceeds 1.5, it is recommended to dilute the sample with the extraction solution Ⅱ before measuring, and multiply the dilution factor in the calculation formula.

2. During the 95°C water bath, the EP tube is tightly closed to prevent water loss. After cooling to room temperature, the next step is performed to avoid liquid splash and burns and affect the test data.

Experimental Examples:

1. Take 0.1 g of Clove leaf and add 1 mL of extract solution for homogenate grinding. After taking the supernatant, operate according to the determination steps. Measured with 96-well flat-bottom plate .Ac=0.358, At=0.566, standard curve line =0.8552x-0. 1864, Ab=0.068， ΔA=At-Ac=0.566-0.358=0.208， x=（ 0.208+0. 1864）÷0.8552=0.461， Calculate enzyme activity based on sample weight:

CWI Activity（U/g weight）= 33.33x÷W=33.33×0.461÷0. 1= 153.711 U/g weight。

Related Products:

AK0285/AK0284 Neutral Invertase(NI) Activity Assay Kit

AK0226/AK0224 Plant Sucrose Content Assay Kit

AK0084/AK0083 Sucrose Synthetase (SS, Cleavage Direction) Activity Assay Kit