Ureide Content In Plants Assay Kit

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

Detection instrument: Spectrophotometer/ Microplate reader

Cat No: BC4375

Size: 100T/48S

Components:

Extract solution A: 60 mL of absolute ethanol, provide for oneself;

Extract solution B: 60 mL × 1 bottle, stored at 4°C;

Reagent 1: 2 mL x 1, stored at 4°C;

Reagent 2: 2 mL × 1, stored at 4°C;

Reagent 3: 2 mL × 1, stored at 4°C;

Reagent 4: 4 mL × 1, stored at 4°C;

Reagent 5: powder ×2, stored at -20°C and protect from light; Just before use, add 4mL of distilled water to dissolve, and store at -20 ℃ for 1 week after dispensing.

Reagent 6: 15 mL of concentrated HCl, provide for oneself;

Reagent 7: 4 mL × 1, stored at 4°C and protect from light;

Standard: powder × 1, 10 mg of allantoin, stored at 4°C and protect from light. Just before use, add 632.5 μL of distilled water to dissolve to prepare a standard solution of 100 μmol/mL.

Product Description:

Breeding legumes with high nitrogen fixation activity is an effective way to improve the nitrogen fixation ability of legumes. The initial output products of soybean rhizobium nitrogen fixation is mainly ureide (allantoin and allantoic acid). The ureide is a nitrogen metabolite in the symbiotic nitrogen fixation of a soybean bacterium. It is the main form of nitrogen storage and transportation and plays an important role in soybean nitrogen metabolism. The nitrogen fixing ability can be evaluated by measuring the content of ureide in legume tissues.

Allantoin is hydrolyzed under peracid or alkaline conditions to generate glyoxylic acid, and then can be oxidized under phenylhydrazine and strong acid conditions to form a red complex with a special absorption peak at 535 nm. The amount of ureide in the sample can be calculated from the absorbance.

Required material

Desk centrifuge, spectrophotometer/microplate reader, water bath/ constant temperature incubator, blast oven, mortar/ homogenizer, micro glass cuvette/96 well plate, 30~50 mesh sieve ,transferpettor, EP tube ice and distilled water.

Procedure:

I. Sample processing:

Plant samples: The plant samples to be tested are dried in a blast oven at 65°C, ground into a powder, and

passed through a 30~50 mesh sieve. By mass (g): the volume of Extract solution(mL) 1: 10 ~ 20 ratio (It is recommended to weigh 0.1 g of dried samples, and add 1.0 mL of extract solution A and 1.0 mL of extract solution B in order. It is prohibited to combine extract solution A and extract solution B mix well for later use), vortex mix, extract in 80°C water bath for 5 min, then centrifugated at 3500 rpm and room temperature for 15 min, discard the precipitate, and take the supernatant on ice for test.

II. Determination procedure:

1 Preheat the spectrophotometer/microplate reader 30min, adjust wavelength to 535nm, set zero with distilled water.

2 Pre-chill reagents 6 and 7 in an ice-water bath for more than 30 minutes, and keep them on ice until use.

3 Treatment of standard solution: After preparing the standard solution as a 100 μmol/mL standard solution, dilute it to 25, 12.5, 6.25, 3.125, 1.5625, 0.78125 nmol/mL standard solution with distilled water for future use..

4 Add reagents with the following list:

Reagent name （μL）	Control tube (C)	Test tube of allantoic acid (TA)	Test tube of ureide (TU)	Standard tube (S)	Blank tube (B)
Sample	80	80	80		
Diluted standard solution				80	
Distilled water					80
Reagent 1	10		10	10	10
Reagent 3	-	20	-		
	-		Thoroughly mix in a boiling water bath for 7 minutes and cool to room temperature.		
Reagent 2	10	-	10	10	10
	-	Mix well, heat in a boiling water bath for 6 minutes, and cool to room temperature.			
Reagent 4	20	20	20	20	20
Reagent 5	20	20	20	20	20
Mix well, let stand for 6 minutes at room temperature, then transfer to an ice-water bath and cool to 4°C.					
Reagent 6	100	100	100	100	100
Reagent 7	20	20	20	20	20
Mix well and let stand for 15 min at room temperature					
Take 200 μL of the reaction solution and measure the absorbance A at 535 nm in a micro glass cuvette/96-well plate, and record them as A_C, A_{TA}, A_{TU}, A_S and A_B. Calculate $\Delta A_{TA}= A_{TA}-A_C$, $\Delta A_{TU}= A_{TU}-A_C$, $\Delta A_S= A_S-A_B$.					

Note: The control tube is not heated with a water bath; Test tube of allantoic acid only needs to be heated with a second boiling water bath; when the test tube, standard tube and blank tube are

heated in a boiling water bath, the EP tube is tightly closed and sealed with a sealing film to avoid liquid evaporation affects test data.

III. Calculation:

1 Standard curve drawing:

Taking the concentration of each standard solution as the x-axis and its corresponding ΔA_S as the y-axis, draw a standard curve to get the standard equation y = kx + b, bring ΔATA and ΔATU into the equation to get x1(nmol/mL)and x2(nmol/mL).

2 Calculation of ureide content in legumes:

Allantoic acid content (nmol / g) = x1 × V_E÷ W.

The ureide content (nmol / g) = x2 × V_E÷ W.

V_E: add extraction volume, 2 mL;

W: sample weight, g.

Note

1. The same batch of test samples need to be equipped with 1-2 blank tubes, standard tubes need only be tested 1-2 times.

2. When the OD value is higher than 1.5, it is recommended to test the sample after dilution and multiply it by the dilution factor in the calculation formula.

3. Reagents 6 and 7 should be pre-chilled on ice for more than 30 minutes before use, and cooled to 0°C.

Experimental Examples:

1. Take 0.1g of Portulaca oleracea, perform sample processing, follow the determination steps, determine by the 96 well plate and calculate ΔA =A t -A c ==0.332-0.226=0.106, bring it into the standard curve y=0.0548x-0.024 , Calculate x2=2.3723, according to the calculation formula:

Ureide Content (nmol/g mass) = 2×x_2÷W=2×2.3723÷0.1=47.446 nmol/g mass.

Related Products:

BC4110/BC4115	Urease(UE) Activity Assay Kit
BC0080/BC0085	Nitrate Reductase(NR) Activity Assay Kit
BC1500/BC1505	Plant Nitrate Nitrogen Activity Assay Kit
BC1520/BC1525	Plant Ammonium Nitrogen Activity Assay Kit
BC1480/BC1485	Soil/Water Nitrite Content Assay Kit