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Ribulose 1, 5-bisphosphate carboxylase/oxygenase(Rubisco) Assay Kit

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

Operation Equipment: Spectrophotometer

Cat No: AK0548

Size:50T/48S

Components:

Extract solution: 50 mL×1. Store at 4℃ .

Reagent I: 50 mL×1. Store at 4℃ .

Reagent II: Powder×1. Store at -20℃ .

Reagent III: Powder×2. Store at -20℃ . Dissolve with 1 mL of distilled water before use;If turbidity appears after oscillation, it can be used after centrifugation.

Reagent IV: Powder×1. Store at -20℃ . Dissolve with 2 mL of distilled water before use.

Working solution: Add all Reagent I to Reagent II before use, mix thoroughly and incubate at 25℃ for 5 minutes.

Product Description:

Ribulose 1,5-bisphosphate carboxylase/oxygenase(Rubisco) is a key enzyme in plant photosynthesis, which controls the carbon dioxide fixation, and restricts the shunt of carbon into the Calvin cycle and photorespiration cycle. The activity of rubisco has direct reflect on the photosynthetic rate.

Rubisco catalyzes combination of one molecule of ribulose- 1,5-diphosphate(RuBP) binds and one molecule of carbon dioxide to produce two molecules of 3-phosphoglycerate (PGA). PGA produces glyceraldehyde-3-phosphate by the action of additional 3-phosphoglycerate kinase and glyceraldehyde-3-phosphate dehydrogenase, which is accompanied by NADH oxidation to form NAD^+. NADH has a characteristic absorption peak at 340 nm, while NAD^+ does not. In this kit, the activity of rubisco is determined by the decrease rate of NADH at 340 nm.

Reagents and Equipment Required but Not Provided:

Ultraviolet spectrophotometer, desk centrifuge, adjustable pipette, water bath, 1 mL quartz cuvette, mortar/homogenizer, ice, distilled water.

Procedure:

I. Sample preparation:

1. Bacteria or cells

Collecting bacteria or cells into the centrifuge tube, after centrifugation discard supernatant. Suggest add 1 mL of Extract solution to 5 million of bacteria or cells. Use ultrasonic to splitting bacteria and cells (placed on ice, ultrasonic power 20%, working time 3 seconds, interval 10 seconds, repeat for 30 times). Centrifuge at 10000 ×g for 10 minutes at 4℃ to remove insoluble materials and take the supernatant on ice

before testing.

2. Tissue

Add 1 mL of Extract solution to 0.1 g of tissue (fresh plant samples are recommended), and fully homogenized on ice bath. Centrifuge at 10000 ×g for 10 minutes at 4℃ to remove insoluble materials, and take the supernatant on ice before testing.

II. Determination procedure:

1. Preheat ultraviolet spectrophotometer for 30 minutes, adjust the wavelength to 340 nm, set zero with distilled water.

2. Add the following reagents

Reagent (μL)	Test tube (T)	Blank tube (B)
Sample	100	-
distilled water	-	100
Reagent III	35	35
Reagent IV	35	35
Working solution	900	900

Detect the absorbance at 340 nm at the time of 20s and 5min20s, record as A1 and A2 respectively. ΔA(T)=A2(T)-A1(T), ΔA(B)= A2(B)-A1(B), ΔA=ΔA(T)-ΔA(B). Kept at 25℃ during the reaction. Blank tube only need to test once or twice.

III. Calculation:

1. Protein concentration:

Unit definition: One unit of enzyme activity is defined as the amount of enzyme catalyzes the production of 1 nmol of NADH per minute every milligram of protein.

Rubisco(U/mg prot)=[ΔA÷(ε×d)×10^9 ×Vrv]÷(Vs×Cpr)÷T=344×ΔA÷Cpr

2. Sample weight:

Unit definition: One unit of enzyme activity is defined as the amount of enzyme catalyzes the production of 1 nmol of NADH per minute every gram of tissue.

Rubisco(U/g weight)=[ΔA÷(ε×d)×10^9 ×Vrv]÷(W÷Ve×Vs)÷T=344×ΔA÷W

3. Bacteria or cultured cells

Unit definition: One unit of enzyme activity is defined as the amount of enzyme catalyzes the production of 1 nmol of NADH per minute every 1 0000 cells or bacteria.

Rubisco(U/10^4 cell)=[ΔA÷(ε×d)×10^9 ×Vrv]÷(Vs÷Ve×500)÷T=0.69×ΔA

ε: NADH molar extinction coefficient, 6.22×10^3 L/mol/cm;

d: Light path of cuvette, 1 cm;

Vrv: Total reaction volume, 1.07×10^{-3} L;

Vs: Supernatant volume, 0.1 mL;

Ve: Extract solution added volume, 1 mL;

Cpr: Sample protein concentration (mg/mL);

T: Reaction time, 5 minutes;
W: Sample weight(g);
500: 5 million cells or bacteria;
10^9: 1 mol =10^9 nmol.

Experimental example:

1. Take 0. 1g of plant leaves, add 1 mL of Extract solution for homogenization, take the supernatant, and then operate according to the determination steps. Measure with micro quartz cuvette and calculate $\Delta A_T = A_{T1}-A_{T2}$=1.279- 1.206=0.073, $\Delta A_B = A_{B1}-A_{B2}$=0.834-0.823=0.011, $\Delta A = \Delta A_T - \Delta A_B$=0.073-0.011=0.062
Rubisco activity (U/g mass) = 344 × ΔA ÷ W =344×0.062÷0. 1=213.28 U/g mass.

Related products:

AK0560/AK0559	Coenzyme Ⅰ NAD(H) Content Assay Kit
AK0500/AK0499	NAD Kinase (NADK) Activity Assay Kit
AK0528/AK0527	NADH Oxidase (NOX) Activity Assay Kit
AK0484/AK0483	NAD Malic Enzyme (NAD-ME) Activity Assay Kit