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

Note: Before the experiment, it is recommended to select 2-3 sample with large expected differences for pre-experiment.

Operation Equipment: Spectrophotometer/Microplate Reader

Catalog Number: BC0445

Size:100T/96S

Product Composition: Before use, please carefully check whether the volume of the reagent is consistent with the volume in the bottle. If you have any questions, please contact Solarbio staff in time.

Reagent name	Size	Preservation Condition
Extract solution	Liquid 100 mL×1	2-8℃
Reagent I	Liquid 25 mL×1	2-8℃
Reagent II	Powder×1	-20℃
Reagent III	Powder×2	-20℃
Reagent IV	Powder×1	-20℃

Solution Preparation:

1. **Reagent III:** Dissolve it with 0.5 mL of distilled water before use; If turbidity appears after oscillation, it can be used after centrifugation (This reagent is a freeze-dried reagent, and there may be significant or even small differences in the amount of reagents observed by the naked eye between different bottles. This phenomenon does not affect the use, and the actual quality is the same.).
2. **Reagent IV:** Dissolve it with 1 mL of distilled water before use.
3. **Working solution:** Add all Reagent I to Reagent II before use, mix thoroughly and incubate at 25℃ for 5 minutes.

Product Description:

1. 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.
2. 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/microplate reader, centrifuge, adjustable pipette, water bath, micro quartz cuvette/96 well UV plate, mortar/homogenizer, ice, and distilled water.

Procedure:

I. Sample preparation:

1. Bacteria or cells

Collecting bacteria or cells into EP 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 200W, working time 3 seconds, interval 10 seconds, repeat for 30 times). Centrifuge at 10000 ×g for 10 minutes at 4℃, and take the supernatant on ice before test.

2. Tissue

Add 1 mL of Extract solution to 0.1 g of tissue, and fully homogenized on ice bath. Centrifuge at 10000 ×g for 10 minutes at 4℃, and take the supernatant on ice before test.

II. Determination procedure:

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

2. Add the following reagents:

Reagent (μL)	Test tube (T)	Blank tube (B)
Sample	20	-
Distilled water	-	20
Reagent III	7	7
Reagent IV	7	7
Working solution	180	180

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

III. Calculation:

A. micro quartz cuvette

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 at 25℃.

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 at 25℃.

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 10000 cells or bacteria at 25℃.

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

Vrv: Total reaction volume, 2.14×10^{-4} L;

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

d: Light path of cuvette, 1 cm;

Vs: Supernatant volume, 0.02 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.

B. 96 well UV plate

The light diameter of 96 well UV plate in the above formula is changed to 0.6 cm for calculation.

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 ΔA(T)= A1(T)-A2(T)=1.2647-1.1825 =0.0822, ΔA(B)=A1(B)–A2(B)= 0.8649-0.8502 = 0.0147, ΔA= ΔA(T) -ΔA(B)= 0.0822-0.0147 = 0.0675
   Rubisco activity (U/g mass) = 344 × ΔA ÷ W = 344 × 0.0675 ÷ 0.1 = 232.2 U/g mass.

Recent Product Citations:

[1] Li Q, Jiang YL, Xia LY, Chen Y, Zhou CZ. Structural insights into cyanobacterial RuBisCO assembly coordinated by two chaperones Raf1 and RbcX. Cell Discov. 2022 Sep 20;8(1):93. doi: 10.1038/s41421-022-00436-9. PMID: 36123352; PMCID: PMC9485235.

[2] Han T, Han X, Ye X, Xi Y, Zhang Y, Guan H. Applying mixotrophy strategy to enhance biomass production and nutrient recovery of Chlorella pyrenoidosa from biogas slurry: An assessment of the mixotrophic synergistic effect. Bioresour Technol. 2022 Dec;366:128185. doi: 10.1016/j.biortech.2022.128185. Epub 2022 Oct 25. PMID: 36307028.

[3] Wang Y, Wang J, Gu Z, Yang S, He Y, Mou H, Sun H. Altering autotrophic carbon metabolism of Nitzschia closterium to mixotrophic mode for high-value product improvement. Bioresour Technol. 2023 Mar;371:128596. doi: 10.1016/j.biortech.2023.128596. Epub 2023 Jan 10.

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[5] Zhang Z, Guo L, Liao Q, Gao M, Zhao Y, Jin C, She Z, Wang G. Bacterial-algal coupling system

for high strength mariculture wastewater treatment: Effect of temperature on nutrient recovery and microalgae cultivation. Bioresour Technol. 2021 Oct;338:125574. doi: 10.1016/j.biortech.2021.125574. Epub 2021 Jul 15. PMID: 34303141.

Reference:

[1] E RINTAMÄKI, E-M ARO. Photosynthetic and Photorespiratory Enzymes in Widely Divergent Plant Species with Special Reference to the Moss Ceratodon purpureus: Properties of Ribulose Bisphosphate Carboxylase/Oxygenase, Phosphoenolpyruvate Carboxylase and Glycolate Oxidase [J]. Journal of Experimental Botany, 1985, 36(11): 1677-1684.

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[3] Pearce FG, Andrews TJ. The relationship between side reactions and slow inhibition of ribulose-bisphosphate carboxylase revealed by a loop 6 mutant of the tobacco enzyme [J]. Journal of Biological Chemistry, 2003, 278(35): 32526-32536.

Related products:

BC0310/BC0315	Coenzyme I NAD(H) Content Assay Kit
BC1030/BC1035	NAD Kinase (NADK) Activity Assay Kit
BC0630/BC0635	NADH Oxidase (NOX) Activity Assay Kit
BC1130/BC1135	NAD Malic Enzyme (NAD-ME) Activity Assay Kit

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