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

Catalog Number: BC0440

Size:50T/48S

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 50 mL×1	2-8℃
Reagent I	Liquid 50 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 1 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 reagent 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 2 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:

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, centrifuge, adjustable pipette, water bath, 1 mL quartz cuvette, 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 1mL 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 (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 test.

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)=A1(T)-A2(T), ΔA(B)=A1(B)-A2(B), ΔA=ΔA(T)-ΔA(B). Maintaining at 25℃ during the reaction process. 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 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,1.07×10^{-3} L;
ε: NADH molar extinction coefficient, 6.22×10^3 L/mol/cm;
d: Light path of cuvette, 1 cm;
Vs: Supernatant volume, 0.1 mL;
Ve: Extract solution volume, 1 mL;
T: Reaction time, 5 minutes;
W: Sample weight(g);
Cpr: Sample protein concentration (mg/mL);
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 ΔA(T)= A1(T)-A2(T)=1.279-1.206=0.073, ΔA(B)=A1(B)–A2(B)=0.834-0.823=0.011, ΔA= ΔA(T)-ΔA(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.

Recent Product Citations:

[1] Li J, Zhao C, Li C, Xue B, Wang S, Zhang X, Yang X, Shen Z, Bo L, He X, Qiu Z, Wang J. Multidrug-resistant plasmid RP4 increases NO and N2O yields via the electron transport system in Nitrosomonas europaea ammonia oxidation. Water Res. 2023 Aug 15; 242:120266. doi: 10.1016/j.watres.2023.120266. Epub 2023 Jul 2. PMID: 37421866.

[2] An J, Fang C, Yuan Z, Hu Q, Huang W, Li H, Ma R, Wang L, Su T, Li S, Wang L, Duan Y, Wang Y, Zhang C, Xu R, Zhang D, Cao Y, Hou J, Kong F, Sun L. A retrotransposon insertion in the Mao1 promoter results in erect pubescence and higher yield in soybean. Proc Natl Acad Sci U S A. 2023 Mar 28;120(13): e2210791120. doi: 10.1073/pnas.2210791120. Epub 2023 Mar 22. PMID: 36947519; PMCID: PMC10068782.

[3] Hou Z, Zhou Q, Xie Y, Mo F, Kang W, Wang Q. Potential contribution of chlorella vulgaris to carbon-nitrogen turnover in freshwater ecosystems after a great sandstorm event. Environ Res. 2023 Oct 1; 234:116569. doi: 10.1016/j.envres.2023.116569. Epub 2023 Jul 7. PMID: 37422116.

[4] Gao P, Guo L, Gao M, Zhao Y, Jin C, She Z. Regulation of carbon source metabolism in mixotrophic microalgae cultivation in response to light intensity variation. J Environ Manage. 2022

Jan 15;302(Pt B):114095. doi: 10.1016/j. jenvman.2021.114095. Epub 2021 Nov 12. PMID: 34775333.

[5] Zhao W, Yang XQ, Zhang QS, Tan Y, Liu Z, Ma MY, Wang MX, Xu B. Photoinactivation of the oxygen-evolving complex regulates the photosynthetic strategy of the seagrass Zostera marina. JPhotochem Photobiol B. 2021 Sep; 222:112259. doi: 10.1016/j.jphotobiol.2021.112259. Epub 2021 Jul 14.

PMID: 34274827.

Reference:

[1] Li J, Zhao C, Li C, Xue B, Wang S, Zhang X, Yang X, Shen Z, Bo L, He X, Qiu Z, Wang J. Multidrug-resistant plasmid RP4 increases NO and N2O yields via the electron transport system in Nitrosomonas europaea ammonia oxidation. Water Res. 2023 Aug 15; 242:120266. doi: 10.1016/j.watres.2023.120266. Epub 2023 Jul 2. PMID: 37421866.

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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