(FOR RESEARCH USE ONLY. DO NOT USE IT IN CLINICAL DIAGNOSIS!)

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

Catalog No.: BC00180

Size: 20T

Please read the instructions carefully before use. If you have any questions or need further help during experiment, please don't hesitate to contact us through the following methods:

✉ Email (Sale) order@enkilife.com

✉ Email (Techsupport) techsupport@enkilife.com

☎ Tel: 0086-27-87002838

Website: www.enkilife.com

Shelf life: Please refer to the label on the outer package.

Techsupport: In order to provide you with better service, please inform us the lot number on the label of the outer package.

Basic Information

Product Name	Ribulose-1,5-bisphosphate carboxylase/oxygenase (Rubisco) Assay Kit
Detection Method	Colorimetric
Sample Type	Plant
Assay Type	Quantitative
Detection Instrument	Spectrophotometer (340 nm)

Product Introduction

Ribulose bisphosphate carboxylase is a key enzyme in plant photosynthesis. It not only controls the fixation of CO_2, but also regulates the distribution of carbon into the Calvin cycle and the photorespiration cycle. Its activity level directly affects the photosynthetic rate.

Principle

Under the catalysis of Rubisco, one molecule of ribulose-1,5-bisphosphate (RuBP) combines with one molecule of CO_2 to produce two molecules of 3-phosphoglyceric acid (PGA). PGA can be converted into glyceraldehyde-3-phosphate by exogenously added phosphoglycerate kinase and glyceraldehyde-3-phosphate dehydrogenase, accompanied by the oxidation of reduced nicotinamide adenine dinucleotide (NADH). Therefore, the oxidation rate of NADH can be calculated from the change in absorbance at 340 nm, which reflects the activity of Rubisco.

Components

No.	Components	Size	Notes
Extraction Solution 1	Liquid	25 mL	Store at 4°C
Extraction Solution 2	Liquid	25 mL	Store at 4°C
Reagent 1	Liquid	30 mL	Store at 4°C

Reagent 2	Powder	1 vial	Store at -20°C. Add 12.5 mL of Reagent 1 immediately before use, mix thoroughly and set aside. Any unused reagent should be aliquoted and stored at -20°C; repeated freeze-thaw cycles are prohibited.
Reagent 3	Powder	1 vial	Store at -20°C. Add 12.5 mL of Reagent 1 immediately before use, mix thoroughly and set aside. Any unused reagent should be aliquoted and stored at -20°C; repeated freeze-thaw cycles are prohibited.
Reagent 4	Powder	1 vial	Store at -20°C. Add 12.5 mL of Reagent 1 immediately before use, mix thoroughly and set aside. Any unused reagent should be aliquoted and stored at -20°C; repeated freeze-thaw cycles are prohibited.

Note: After Reagent 2, 3 and 4 are dissolved, aliquot them as needed and store at -20°C.

Storage

The kit has a validity period of 3 months.

Preparation

- **Sample handling**

1. **Extraction of total Rubisco enzyme**: Weigh approximately 0.1 g of sample and add 1 mL of Extraction Solution 1. Homogenize the sample in an ice bath, followed by ultrasonic fragmentation (ice bath condition, 200 W; sonication for 3 s with a 7 s interval, total duration: 1 min). Then centrifuge at 8000 g and 4 °C for 10 min, and collect the supernatant for subsequent determination.
2. **Separation of cytoplasmic and chloroplastic Rubisco enzyme**: Prepare the system at a

mass-to-volume ratio of plant tissue (g) to extract solution (mL) of 1:5–10 (it is recommended to weigh about 0.1 g of tissue and add 1 mL of Extraction Solution I). After ice-bath homogenization, centrifuge at 200 g and 4 °C for 5 min. Separate and retain the precipitate, and collect the supernatant. Further centrifuge the supernatant at 8000 g and 4 °C for 10 min; the resulting supernatant is used for the determination of cytoplasmic Rubisco activity. Resuspend the precipitate with 1 mL of Extraction Solution II, dissolve by shaking, and perform ultrasonic fragmentation (ice bath, 200 W; sonication for 3 s with a 7 s interval, total duration: 1 min). Afterwards, centrifuge at 8000 g and 4 °C for 10 min, and collect the supernatant to measure chloroplastic Rubisco activity.

It is recommended to determine the total Rubisco activity and extract the crude enzyme solution according to Step 1. If it is necessary to separately measure Rubisco in the cytoplasm and chloroplasts, extract the crude enzyme solution following Step 2.
(Note: Ultrasonic fragmentation shall be performed with a cell disruptor during the preparation of crude enzyme solution.)

Operation process

Before formal determination, be sure to select 2-3 samples with expected large differences to conduct a preliminary experiment.

1. Preheat the spectrophotometer for at least 30 min, adjust the wavelength to 340 nm, and zero the instrument with distilled water.
2. Preparation of working solution: Mix Reagent 2 and Reagent 3 at a ratio of 1:1 immediately before use, and prepare only the required amount.
3. Operation table:

	Assay tube
Sample to be tested (μL)	50
Reagent 4 (μL)	50
Working solution (μL)	900

Add the above reagents into a 1 mL cuvette, mix thoroughly, record the absorbance A_1 at 20 s and absorbance A_2 at 5 min 20 s at 340 nm, and calculate $\Delta A = A_1 - A_2$ (the reaction time is 5 minute).

Calculation

a. Calculated based on the protein concentration of the sample.

Definition: One enzyme activity unit is defined as the amount of enzyme that oxidizes 1 nmol of NADH per minute per milligram of protein at 25 ℃.

Calculation formula:

$$\text{Rubisco}((\text{nmol/min})/\text{mg}) = [\Delta A \times V_{\text{Total}} \div (\varepsilon \times d) \times 10^9] \times (V_{\text{Sample}} \times \text{Cpr}) \div T$$

b. Calculated based on sample fresh weight.

Definition: One enzyme activity unit is defined as the oxidation of 1 nmol NADH per minute per gram of tissue at 25 ℃.

Calculation formula:

$$\text{Rubisco}((\text{nmol/min})/\text{g}) = [\Delta A \times V_{\text{Total}} \div (\varepsilon \times d) \times 10^9] \times \left(\frac{V_{\text{Sample}}}{V_{\text{Extraction Solution}}} \times W\right) \div T$$

Annotation:

V_{Total}: Total volume of reaction system, 1×10^{-3} L

V_{Sample}: Volume of sample added, 0.05 mL

$V_{\text{Extraction Solution}}$: Volume of extraction solution added, 1 mL

ε: Molar extinction coefficient of NADH, 6.22×10^3 L/mol/cm

d: Optical path length of cuvette, 1 cm

T: Reaction time, 5 min

Cpr: Sample protein concentration, mg/mL

W: Sample mass, g

Precautions

1. It is recommended to use disposable laboratory test tubes for operation.
2. This product is intended for scientific research use only by professionals and must not be used for clinical diagnosis or treatment, in food or drugs, or stored in ordinary residences.