Ribulose Bisphosphate Carboxylase/Oxygenase (Rubisco) Colorimetric Assay Ki

Cat#: orb1173179 (User Manual)

Micro Ribulose Bisphosphate Carboxylase/Oxygenase (Rubisco) Assay Kit

Size: 48 T/96 T
Lot #: Refer to product label
Applicable samples: Plant Tissues
Storage: Stored at -20℃ for 6 months, protect from light

Assay Principle

1, 5-ribulose bisphosphate carboxylase/oxygenase (Rubisco, EC 4.1.1.39) is a key enzyme in plant photosynthesis, which not only controls the fixation of CO2, but also controls the distribution of carbon to the Calvin cycle and the photorespiration cycle. Its activity directly affects the photosynthetic rate. CheKine™ Micro Ribulose Bisphosphate Carboxylase/Oxygenase (Rubisco)

Assay Kit provides a simple method for detecting Rubisco activity in plant tissues. The principle is one molecule of ribulose-1,5-diphosphate (RuBP) combines with one molecule of CO2 to produce two molecules of 3-phosphoglyceric acid (PGA).

Glyceraldehyde-3-phosphate can be produced by PGA through the action of additional 3-phosphoglycerate kinase and glyceraldehyde-3-phosphate dehydrogenase, which is accompanied by NADH oxidation to produce NAD+. NADH has a characteristic absorption peak at 340 nm, while NAD+ does not. Rubisco activity is calculated by measuring the rate of decrease in light absorption at 340 nm.

Materials Supplied and Storage Conditions

Kit components	Size		Storage conditions
	48 T	96 T	
Extraction Buffer Ⅰ	50 mL	100 mL	4℃
Extraction Buffer Ⅱ	50 mL	100 mL	4℃
Reagent Ⅰ	12.5 mL	25 mL	4℃
Reagent Ⅱ	1	1	-20℃, protect from light
Reagent Ⅲ	1	1	-20℃, protect from light
ReagentⅣ	1	1	-20℃, protect from light

Materials Required but Not Supplied

·Microplate reader or ultraviolet spectrophotometer capable of measuring absorbance at 340 nm
·96-well UV plate or microquartz cuvette, precision pipettes, disposable pipette tips
·Ice maker, refrigerated centrifuge, incubator
·Deionized water
·Homogenizer (for tissue samples)

Reagent Preparation

Extraction Buffer Ⅰ: Ready to use as supplied. Equilibrate to room temperature before use. Store at 4℃.
Extraction Buffer Ⅱ: Ready to use as supplied. Equilibrate to room temperature before use. Store at 4℃.
Reagent Ⅰ: Ready to use as supplied. Equilibrate to room temperature before use. Store at 4℃.
Working Reagent Ⅱ: Before use, add 5 mL Reagent Ⅰ to dissolve for 48 T; add 10 mL Reagent Ⅰ to dissolve for 96 T, mix well for use. The remaining reagents should be stored at -20℃ and protected from light after aliquoting to avoid repeated freezing and thawing.
Working ReagentⅢ: Before use, add 5 mL Reagent Ⅰ to dissolve for 48 T; add 10 mL Reagent Ⅰ to dissolve for 96 T, mix well for use. The remaining reagents should be stored at -20℃ and protected from light after aliquoting to avoid repeated freezing and thawing.
Working ReagentⅣ: Before use, add 0.5 mL Reagent Ⅰ to dissolve for 48 T; add 1 mL Reagent Ⅰ to dissolve for 96 T, mix well for use. The remaining reagents should be stored at -20℃ and protected from light after aliquoting to avoid repeated freezing and thawing.
Working Solution: Before use, mix Working Reagent Ⅱ and Working Reagent Ⅲ in a ratio of 1:1, and prepare the corresponding volume according to the experimental requirements.

Sample Preparation

Preparation of crude enzyme solution:
1. Extraction of total Rubisco enzyme: Weigh 0.1 g samples, add 1 mL Extraction Buffer Ⅰ, homogenize in ice. Ultrasonic break in ice 1 min (power 20% or 200 W, ultrasonic 3 s, interval 7 s). Centrifuge at 8,000 g for 10 min at 4℃. Use supernatant for assay, and place it on ice to be tested.
2. Separation of cytoplasm and chloroplast Rubisco enzyme: Weigh 0.1 g samples, add 1 mL Extraction Buffer Ⅰ, homogenize in ice, centrifuge at 200 g at 4℃ for 5 min, discard the precipitate, take the supernatant and centrifuge at 8,000 g for 10 min at 4℃, take the supernatant after centrifugation to determine the cytoplasmic Rubisco enzyme activity, add 1 mL of Extraction Buffer Ⅱ to the precipitate, shake and dissolve, and ultrasonic break in ice 1 min (power 20% or 200 W, ultrasonic 3 s, interval 7 s). Centrifuge at 8,000 g for 10 min at 4℃, and take the supernatant to determine the Rubisco enzyme activity in chloroplasts.

Note: It is recommended to determine the total Rubisco enzyme activity and extract the crude enzyme solution according to step 1. If you need to determine the Rubisco in the cytoplasm and chloroplast separately, follow step 2 to extract the crude enzyme solution. It will be better to quantify the total protein with Protein Quantification Kit (BCA Assay), if the content is calculated by protein concentration.

Assay Procedure

1. Preheat the microplate reader or ultraviolet spectrophotometer for more than 30 min, and adjust the wavelength to 340 nm, ultraviolet spectrophotometer was returned to zero with deionized water.
2. Sample measurement (the following operations are operated in the 96-well UV plate or microquartz cuvette).

Reagent	Blank Well (μL)	Test Well (μL)
Sample	0	10
Deionized Water	10	0
Working Reagent IV	10	10
Working Solution	180	180

3. After mixing quickly, record the absorbance values of 20 s and 5 min 20 s at 340 nm with a microplate reader. The test well is marked as A1 and A2, and the blank well is marked as A3 and A4. Finally calculate ΔATest=(A1-A2)-(A3-A4).

Note: Blank well only needs to measure 1 time. In order to guarantee the accuracy of experimental results, need to do a pre-experiment with 2-3 samples. If the absorbance value of the sample is not within the measurement range, dilute or increase the sample quantity appropriately.

Data Analysis
Note: We provide you with calculation formulae, including the derivation process and final formula. The two are exactly equal. It is suggested that the concise calculation formula in bold is final formula.

A. 96-well UV plates calculation formula as below
(1) Calculation according to the protein concentration of the sample
Unit definition: an enzyme activity unit defines as 1 mg tissue protein catalyzes the oxidation of 1 nmol NADH per min in the reaction system at 25℃.
Rubisco (U/mg prot)=[ΔATest×VTotal÷(ε×d)×109]÷(VSample×Cpr)÷T**=1,286×ΔATest÷Cpr**

(2) Calculation according to the weight of the sample
Unit definition: an enzyme activity unit defines as 1 g tissues catalyzes the oxidation of 1 nmol NADH per min in the reaction system at 25℃.
Rubisco (U/g fresh weight)=[ΔATest×VTotal÷(ε×d)×109]÷(W÷VExtraction×VSample)÷T**=1,286×ΔATest÷W**
Where: VTotal: the total volume of the reaction system, 0.2mL=2×10-4 L, VExtraction: the volume of the Extraction Buffer, 1 mL; VSample: the volume of the supernatant in the reaction system, 0.01 mL; ε: NADH molar extinction coefficient, 6.22×103 L/mol/cm; d: 96-well
UV plate diameter, 0.5 cm; Cpr: protein concentration (mg/mL); T: reaction time, 5 min; W: sample weight, g.
B. Microquartz cuvette calculation formula
The optical diameter d: 0.5 cm in the above calculation formula can be adjusted to d: 1 cm for calculation.

Typical Data

Weigh 0.1 g of arabidopsis thaliana leaves and add 1 mL Extraction Buffer Ⅰ for homogenization and grinding, take supernatant, then follow the determination steps, and measure with 96-well UV plate:
A1=0.918, A2=0.842, A3=0.8063, A4=0.863, ΔATest=(A1-A2)-(A3-A4)=(0.918-0.842)-(0.863-0.863)=0.076, calculate the Rubisco activity according to the weight of the sample:
Rubisco (U/g weight)=1,286×ΔATest÷W=1,286×0.076÷0.1=977.36 U/g.