

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

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

**Operation Equipment:** Spectrophotometer/Microplate Reader

**Cat No:** AK0547

**Size:**100T/96S

### **Components:**

Extract solution: 100 mL×1. Store at 4°C .

Reagent I: 25 mL×1. Store at 4°C .

Reagent II: Powder×1. Store at -20°C .

Reagent III: Powder×2. Store at -20°C . Dissolve with 0.5 mL of distilled water before use; If turbidity appears after oscillation, it can be used after centrifugation.

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

**Working solution:** Add all Reagent I to Reagent II before use, mix thoroughly and incubate at 25°C 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<sup>+</sup>. NADH has a characteristic absorption peak at 340 nm, while NAD<sup>+</sup> 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, desk centrifuge, adjustable pipette, water bath, micro quartz cuvette/96 well flat-bottom UV plate, 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°C to remove insoluble materials 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°C to remove insoluble materials, 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, 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 320s, record as A1 and A2 respectively.  $\Delta A(T) = A2(T) - A1(T)$ ,  $\Delta A(B) = A2(B) - A1(B)$ ,  $\Delta A = \Delta A(T) - \Delta A(B)$ . Keep at 25°C during the reaction. 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.

$$\text{Rubisco (U/mg prot)} = [\Delta A \div (\epsilon \times d) \times 10^9 \times V_{rv}] \div (V_s \times C_{pr}) \div T = 344 \times \Delta A \div C_{pr}$$

#### 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.

$$\text{Rubisco (U/g weight)} = [\Delta A \div (\epsilon \times d) \times 10^9 \times V_{rv}] \div (W \div V_e \times V_s) \div T = 344 \times \Delta A \div W$$

#### 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.

$$\text{Rubisco (U/10}^4 \text{ cell)} = [\Delta A \div (\epsilon \times d) \times 10^9 \times V_{rv}] \div (V_s \div V_e \times 500) \div T = 0.69 \times \Delta A$$

$\epsilon$ : NADH molar extinction coefficient,  $6.22 \times 10^3$  L/mol/cm;

d: Light path of cuvette, 1 cm;

$V_{rv}$ : Total reaction volume,  $2.14 \times 10^{-4}$  L;

$V_s$ : Supernatant volume, 0.02 mL;

$V_e$ : 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 flat-bottom UV plate**

The light diameter of d-96 well flat-bottom 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  $\Delta A_T = A_{T1} - A_{T2} = 1.2647 - 1.1825 = 0.0822$ ,  $\Delta A_B = A_{B1} - A_{B2} = 0.8649 - 0.8502 = 0.0147$ ,  $\Delta A = \Delta A_T - \Delta A_B = 0.0822 - 0.0147 = 0.0675$

Rubisco activity (U/g mass) =  $344 \times \Delta A \div W = 344 \times 0.0675 \div 0.1 = 232.2$  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