RuBisCO Activity

Colorimetric Microplate Assay Kit

User Manual

Catalog # CAK1143

(Version 2.3B)

Detection and Quantification ofRuBisCOActivityinTissue extracts,

Cell lysate, Cell culture media and Other biological fluidsSamples.

For research use only. Not for diagnostic or therapeutic procedures.

I. INTRODUCTION

Ribulose-1,5-bisphosphate carboxylase/oxygenase (EC 4.1.1.39), commonly known by the abbreviations RuBisCO, RuBPCase, or RuBPco, is an enzyme involved in the first major step of carbon fixation, a process by which atmospheric carbon dioxide is converted by plants and other photosynthetic organisms to energy-rich molecules such as glucose. In chemical terms, it catalyzes the carboxylation of ribulose-1,5-bisphosphate (also known as RuBP). It is probably the most abundant enzyme on Earth.

RuBisCO Activity Colorimetric Microplate Assay Kit is a sensitive assay for determining RuBisCO activity in various samples. RuBisCO activity is determined by NADH decomposition rate. The reaction products can be measured at a colorimetric readout at 450 nm.

II.KIT COMPONENTS

Component	Volume	Storage
96-WellMicroplate	1 plate	
Assay Buffer	30 mlx 4	4 °C
Reaction Buffer	10 mlx 1	4 °C
Substrate	Powder x 1	-20 °C
Enzyme I	10 µlx 1	4 °C
Enzyme II	Powder x 1	-20 °C
Dye ReagentA	Powder x 1	4 °C
Dye Reagent B	1 ml x 1	4 °C
Standard	Powder x 1	-20 °C
Plate Adhesive Strips	3 Strips	
Technical Manual	1 Manual	

III. MATERIALS REQUIRED BUT NOT PROVIDED

1. Microplate reader to read absorbance at 450 nm
2. Distilled water
3. Pipettor, multi-channel pipettor
4. Pipette tips
5. Mortar
6. Centrifuge
7. Timer
8. Ice

IV. REAGENT PREPARATION

Substrate: Briefly centrifuge prior to opening. Add 1 ml Reaction Bufferto dissolve before use.Store at -80 °C for 1 week.

Enzyme I: add 1 ml Reaction Bufferto dissolve before use.Store at -80 °C for 1 week.

Enzyme II: add 1 ml Reaction Bufferto dissolve before use.Store at -80 °C for 1 week.

Dye Reagent A: add 9 mldistilled water to dissolve before use, mix, store at 4°C.

Standard:Briefly centrifuge prior to opening, add 1 mldistilled water to dissolve before use; then add 0.2 ml into 0.8 mldistilled water, the concentration will be 400 µmol/L.Then perform 2-fold serial dilutions of the top solution using distilled waterto make the standard curve. The concentration of standard curve could be 400/200/100/50/25/12.5/6.25µmol/L.Store at -20 °C for 1 weekor 4°C for 1 day.

V. SAMPLE PREPARATION

1.For cell and bacteria samples

Collect cell or bacteria into centrifuge tube, discard the supernatant after centrifugation, add 1 mlAssay Buffer for 5 ×10^6 cell or bacteria, sonicate (with power 20%, sonicate 3s, interval 10s,repeat 30 times); centrifuged at 10,000g 4°C for 15 minutes, take the supernatant into a new centrifuge tube and keep it on ice for detection.

2.For tissue samples

Weigh out 0.1 g tissue, homogenize with 1 mlAssay Buffer on ice, centrifuged at 10,000g 4°C for 10 minutes, take the supernatant into a new centrifuge tube and keep it on ice for detection.

3.For liquid samples

Add 0.9 mlAssay Buffer into 0.1 ml liquid sample, centrifuged at 10,000g 4°C for 10minutes, take the supernatant into a new centrifuge tube and keep it on ice for detection.

VI. ASSAY PROCEDURE

Warm all reagents to room temperature before use.

Add following reagents intothe microplate:

Reagent*	Sample**	Control	Standard	Blank
Reaction Buffer	60 µl	60 µl	--	--
Sample	10 µl	--	--	--
Standard	--	--	100 µl	--
Distilled water	--	10 µl	--	100 µl
Enzyme I	10 µl	10 µl	--	--
Enzyme II	10 µl	10 µl	--	--
Substrate	10 µl	10 µl	--	--
Mix, incubate at 37 °C for 30 minutes				
Dye Reagent A	90 µl	90 µl	90 µl	90 µl
Dye Reagent B	10 µl	10 µl	10 µl	10 µl
Mix, keep for 5 minutes at room temperature, record absorbance measured at 450 nm.				

Note:

*Reagents must be added sequentially and should not be premixed prior to addition.

**The concentrations can vary over a wide range depending on the different samples. For unknown samples, we recommend doing a pilot experiment & testing several doses to ensure the readings are within the standard curve range.

**For colored samples, we recommend setting a parallel sample background control well with same volume of sample only. Other reagents were replaced by distilled water to the same total volume. Subtract the OD value of the sample background control from the OD value of the sample to correct for interference from the sample's own color.

VII. CALCULATION

Unit Definition: One Unit ofRuBisCO activity is defined as the enzyme that reduces 1 μmol of NADH per minute.

1. According to the slope of the standard curve

$$\text{Activity} = \frac{(OD_{Control}-OD_{Sample}) - \text{Intercept}}{\text{Slope}\times T} \times \frac{V_{Standard}}{V_{Sample}} \times n(U/mL)$$

2. According to one point of the standard OD value and concentration

2.1According to the quantity of cells

$$\text{Activity} = \frac{(C_{Standard}\times V_{Standard}) \times (OD_{Control}-OD_{Sample})}{(OD_{Standard} - OD_{Blank}) \times N \times (V_{Sample}/ V_{Assay}) \times T}(U/10^4)$$

2.2 According to the weight of sample

$$\text{Activity} = \frac{(C_{Standard}\times V_{Standard}) \times (OD_{Control}-OD_{Sample})}{(OD_{Standard} - OD_{Blank}) \times W \times (V_{Sample}/ V_{Assay}) \times T}(U/g)$$

2.3 According to the volume of sample

$$\text{Activity} = \frac{(C_{Standard}\times V_{Standard}) \times (OD_{Control}-OD_{Sample})}{(OD_{Standard} - OD_{Blank}) \times V_{Sample}\times T}(U/ml)$$

Slope: the absorbance slope of standard curve

n: the dilution factor

W: the weight of total sample, g

N: the quantity of total cell sample, 10^4

$C_{Standard}$: the concentration of standard, μmol/mL

$V_{Standard}$: the volume of standard in assay procedure, mL

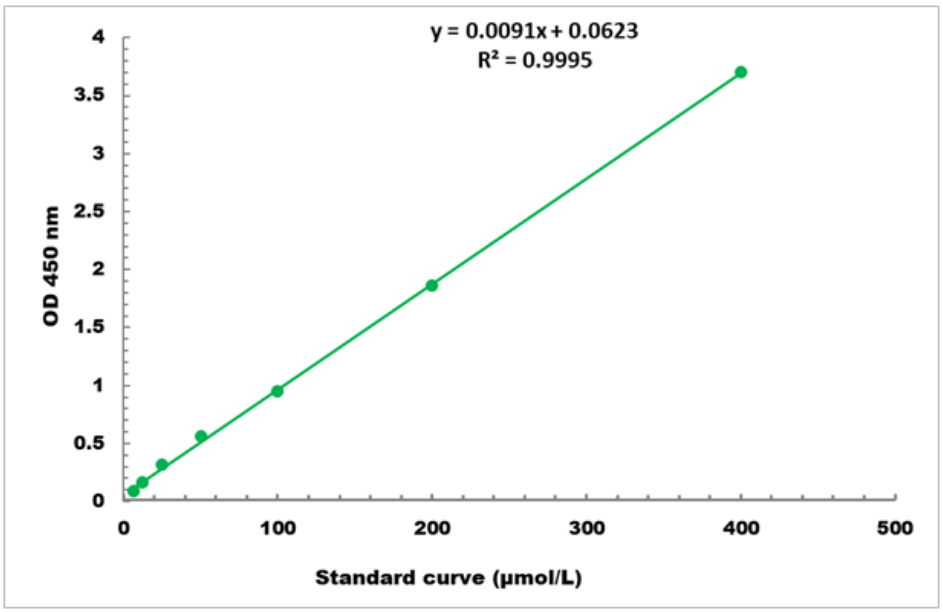
V_{Sample}: the volume of sample in assay procedure, mL

V_{Assay}: the volume of Assay Buffer in sample preparation, mL

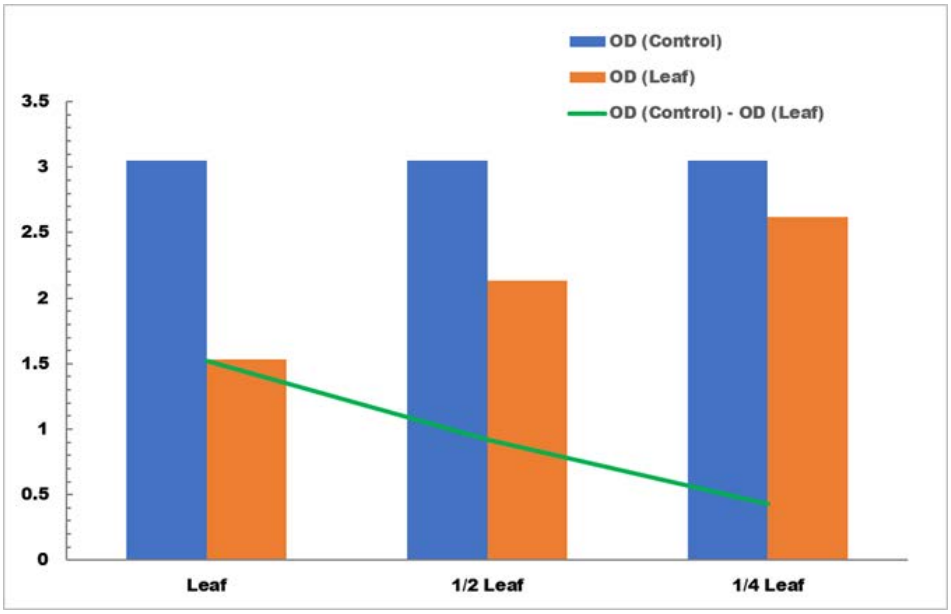
T: the reaction time, minute

VIII. TYPICAL DATA

The standard curve is for demonstration only. A standard curve must be run with each assay.



Detection Range: 4µmol/L - 400 µmol/L



Determination of RuBisCO in leaf