RuBisCO Microplate Assay Kit

User Manual

Catalog # CAK1143

(Version 1.3A)

Detection and Quantification of RuBisCO Activity in Tissue extracts, Cell lysate, Cell culture media and Other biological fluids Samples.

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 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 340 nm.

II. KIT COMPONENTS

Component	Volume	Storage
96-Well Microplate	1 plate	
Assay Buffer	30 ml x 4	4 °C
Diluent	20 ml x 1	4 °C
Substrate	Powder x 1	4 °C
Enzyme	Powder x 1	-20 °C
Standard	Powder x 1	-20 °C
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Note:

Substrate: add 18 ml Diluent to dissolve before use.

Enzyme: add 1 ml Diluent to dissolve before use.

Standard: add 1 ml distilled water to dissolve before use; then add 0.2 ml into 0.8 ml distilled water, the concentration will be 400 µmol/L.

III. MATERIALS REQUIRED BUT NOT PROVIDED

1. Microplate reader to read absorbance at 340 nm
2. Distilled water
3. Pipettor, multi-channel pipettor
4. Pipette tips
5. Mortar
6. Centrifuge
7. Timer
8. Ice

IV. SAMPLE PREPARATION

1. For cell and bacteria samples

Collect cell or bacteria into centrifuge tube, discard the supernatant after centrifugation, add 1 ml Assay 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 ml Assay 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 ml Assay buffer into 0.1 ml liquid sample, centrifuged at 10,000g 4 °C for 10 minutes, take the supernatant into a new centrifuge tube and keep it on ice for detection.

V. ASSAY PROCEDURE

Warm all reagents to room temperature before use.

Add following reagents into the microplate:

Reagent	Sample	Standard	Blank
Standard	--	200 µl	--
Distilled water	--	--	200 µl
Enzyme	10 µl	--	--
Substrate	180 µl	--	--
Sample	10 µl	--	--
Mix, measured at 340 nm and record the absorbance of 10th second and 130th second.			

Note:

1) Perform 2-fold serial dilutions of the top standards to make the standard curve.

2) For unknown samples, we recommend doing a pilot experiment & testing several doses to ensure the readings are within the standard curve range. If the enzyme activity is lower, please add more sample into the reaction system; or increase the reaction time; if the enzyme activity is higher, please dilute the sample, or decrease the reaction time.

3) Reagents must be added step by step, can not be mixed and added together.

VI. CALCULATION

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

1. According to the protein concentration of sample

$$\text{RuBisCO (U/mg)} = (C_{Standard} \times V_{Standard}) \times (OD_{Sample(10S)} - OD_{Sample(130S)}) / (OD_{Standard} - OD_{Blank}) / (V_{Sample} \times C_{Protein}) / T$$

$$= 4 \times (OD_{Sample(10S)} - OD_{Sample(130S)}) / (OD_{Standard} - OD_{Blank}) / C_{Protein}$$

2. According to the weight of sample

$$\text{RuBisCO (U/g)} = (C_{Standard} \times V_{Standard}) \times (OD_{Sample(10S)} - OD_{Sample(130S)}) / (OD_{Standard} - OD_{Blank}) / (V_{Sample} \times W / V_{Assay}) / T$$

$$= 4 \times (OD_{Sample(10S)} - OD_{Sample(130S)}) / (OD_{Standard} - OD_{Blank}) / W$$

3. According to the quantity of cells or bacteria

$$\text{RuBisCO (U/10}^4\text{)} = (C_{Standard} \times V_{Standard}) \times (OD_{Sample(10S)} - OD_{Sample(130S)}) / (OD_{Standard} - OD_{Blank}) / (V_{Sample} \times N / V_{Assay}) / T$$

$$= 4 \times (OD_{Sample(10S)} - OD_{Sample(130S)}) / (OD_{Standard} - OD_{Blank}) / N$$

4. According to the volume of sample

$$\text{RuBisCO (U/ml)} = (C_{Standard} \times V_{Standard}) \times (OD_{Sample(10S)} - OD_{Sample(130S)}) / (OD_{Standard} - OD_{Blank}) / V_{Sample} / T$$

$$= 4 \times (OD_{Sample(10S)} - OD_{Sample(130S)}) / (OD_{Standard} - OD_{Blank})$$

$C_{Standard}$: the standard concentration, 400 μmol/L = 0.4 μmol/ml;

$V_{Standard}$: the volume of standard, 200 μl = 0.2 ml;

$C_{Protein}$: the protein concentration, mg/ml;

W: the weight of sample, g;

N: the quantity of cell or bacteria, N × 10^4;

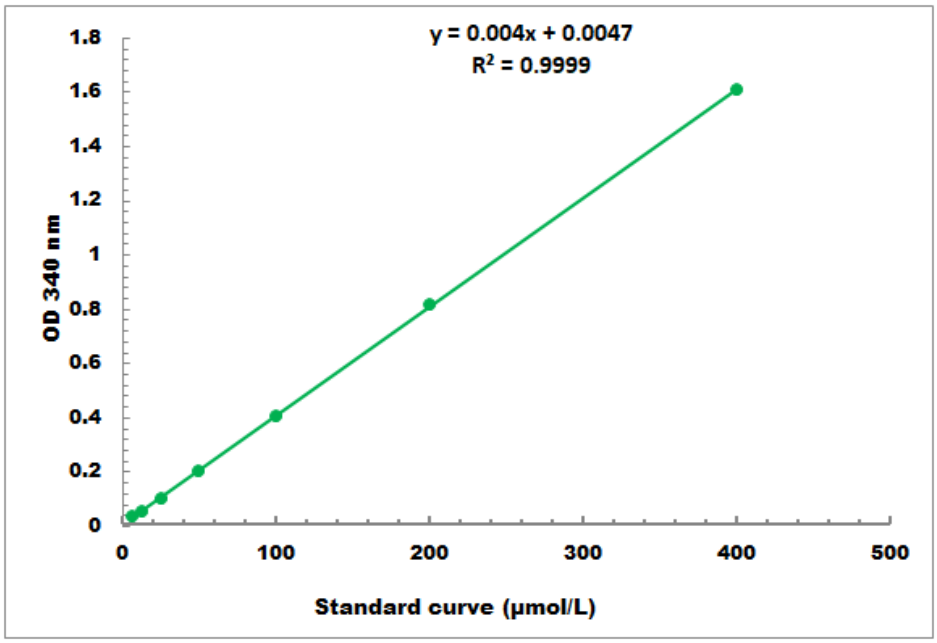
V_{Sample}: the volume of sample, 0.01 ml;

V_{Assay}: the volume of Assay buffer, 1 ml;

T: the reaction time, 2 minutes.

VII. TYPICAL DATA

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



Detection Range: 4 μmol/L - 400 μmol/L

VIII. TECHNICAL SUPPORT

For troubleshooting, information or assistance, please go online to www.cohesionbio.com or contact us at techsupport@cohesionbio.com

IX. NOTES