



# **RuBisCO Microplate Assay Kit**

## **User Manual**

**Catalog # FTA0135**

(Version 1.2A)

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.**

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## 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  $5 \times 10^6$  cell or bacteria, sonicate (with power 20%, sonication 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 $\mu$ l	--
Distilled water	--	--	200 $\mu$ l
Enzyme	10 $\mu$ l	--	--
Substrate	180 $\mu$ l	--	--
Sample	10 $\mu$ l	--	--
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.

## VI. CALCULATION

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

### 1. According to the protein concentration of sample

$$\begin{aligned}\text{RuBisCO (U/mg)} &= (C_{\text{Standard}} \times V_{\text{Standard}}) \times (\text{OD}_{\text{Sample}(10\text{S})} - \text{OD}_{\text{Sample}(130\text{S})}) / (\text{OD}_{\text{Standard}} - \\ &\quad \text{OD}_{\text{Blank}}) / (V_{\text{Sample}} \times C_{\text{Protein}}) / T \\ &= 4000 \times (\text{OD}_{\text{Sample}(10\text{S})} - \text{OD}_{\text{Sample}(130\text{S})}) / (\text{OD}_{\text{Standard}} - \text{OD}_{\text{Blank}}) / C_{\text{Protein}}\end{aligned}$$

### 2. According to the weight of sample

$$\begin{aligned}\text{RuBisCO (U/g)} &= (C_{\text{Standard}} \times V_{\text{Standard}}) \times (\text{OD}_{\text{Sample}(10\text{S})} - \text{OD}_{\text{Sample}(130\text{S})}) / (\text{OD}_{\text{Standard}} - \\ &\quad \text{OD}_{\text{Blank}}) / (V_{\text{Sample}} \times W / V_{\text{Assay}}) / T \\ &= 4000 \times (\text{OD}_{\text{Sample}(10\text{S})} - \text{OD}_{\text{Sample}(130\text{S})}) / (\text{OD}_{\text{Standard}} - \text{OD}_{\text{Blank}}) / W\end{aligned}$$

### 3. According to the quantity of cells or bacteria

$$\begin{aligned}\text{RuBisCO (U/10}^4\text{)} &= (C_{\text{Standard}} \times V_{\text{Standard}}) \times (\text{OD}_{\text{Sample}(10\text{S})} - \text{OD}_{\text{Sample}(130\text{S})}) / (\text{OD}_{\text{Standard}} - \\ &\quad \text{OD}_{\text{Blank}}) / (V_{\text{Sample}} \times N / V_{\text{Assay}}) / T \\ &= 4000 \times (\text{OD}_{\text{Sample}(10\text{S})} - \text{OD}_{\text{Sample}(130\text{S})}) / (\text{OD}_{\text{Standard}} - \text{OD}_{\text{Blank}}) / N\end{aligned}$$

### 4. According to the volume of sample

$$\begin{aligned}\text{RuBisCO (U/ml)} &= (C_{\text{Standard}} \times V_{\text{Standard}}) \times (\text{OD}_{\text{Sample}(10\text{S})} - \text{OD}_{\text{Sample}(130\text{S})}) / (\text{OD}_{\text{Standard}} - \\ &\quad \text{OD}_{\text{Blank}}) / V_{\text{Sample}} / T \\ &= 4000 \times (\text{OD}_{\text{Sample}(10\text{S})} - \text{OD}_{\text{Sample}(130\text{S})}) / (\text{OD}_{\text{Standard}} - \text{OD}_{\text{Blank}})\end{aligned}$$

$C_{\text{Standard}}$ : the standard concentration, 400  $\mu\text{mol/L}$  = 400 nmol/ml;

$V_{\text{Standard}}$ : the volume of standard, 200  $\mu\text{l}$  = 0.2 ml;

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

W: the weight of sample, g;

N: the quantity of cell or bacteria,  $N \times 10^4$ ;

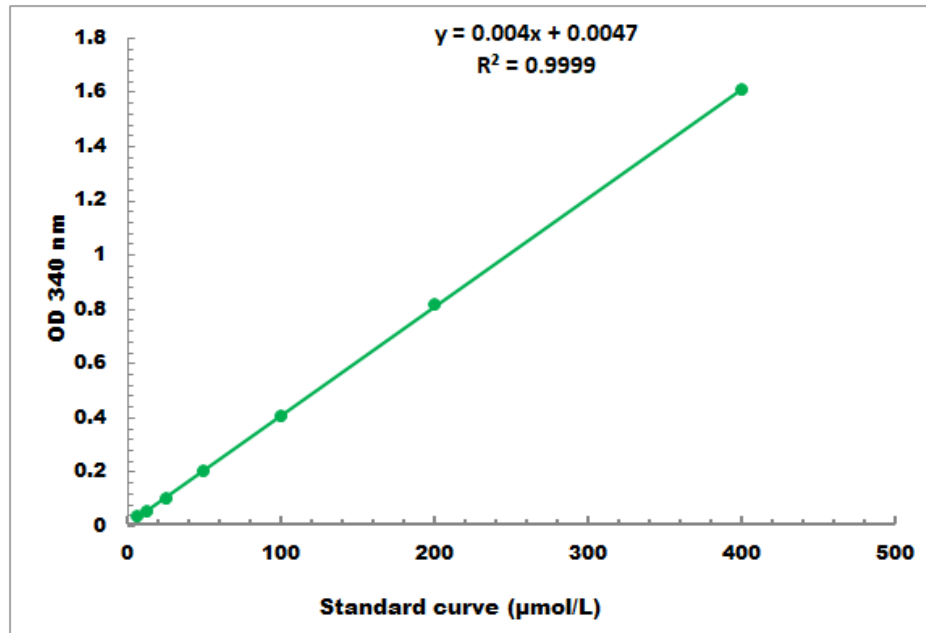
$V_{\text{Sample}}$ : the volume of sample, 0.01 ml;

$V_{\text{Assay}}$ : 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](http://www.cohesionbio.com) or contact us at [techsupport@cohesionbio.com](mailto:techsupport@cohesionbio.com)

## IX. NOTES