



Cinnamate 4-hydroxylase Activity Colorimetric Microplate Assay Kit User Manual

Catalog # CAK1196

(Version 1.5A)

Detection and Quantification of Cinnamate 4-hydroxylase
(C4H) 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

The phenylpropanoid pathway is the specific secondary metabolism in plants, and belongs to one of the three plant's secondary metabolism pathways. In vascular plants, the phenylpropanoid's final product is used to synthesize lignin monomer, and the flavonoid which plays an important role in pigment formation, medicinal ingredients, and the interaction between microbes and plants. Cinnamate 4-hydroxylase, which has an important function and catalyzes the second step in phenylpropanoid pathway, to be specific, it converts the trans-cinnamic acid into p-cinnamic acid.

Cinnamate 4-hydroxylase Activity Colorimetric Microplate Assay Kit is a sensitive assay for determining cinnamate 4-hydroxylase activity in various samples. The color intensity, measured at 340 nm, is proportionate to the enzyme activity in the sample.

II.KIT COMPONENTS

Component	Volume	Storage
96-Well Microplate	1 plate	
Assay Buffer I	30 ml x 4	4 °C
Assay Buffer II	Powder x 1	4 °C
Substrate	Powder x 1	-20 °C
Reaction Buffer	20 ml x 1	4 °C
Standard	Powder x 1	-20 °C
Technical Manual	1 Manual	

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. Ice
7. Centrifuge
8. Timer

IV. REAGENT PREPARATION

Standard: Briefly centrifuge prior to opening. Dissolve in 1 ml distilled water to generate 2 mmol/L of standard stock solution, store at 4 °C for 1 day or -20°C for 3 days after reconstitution. Then dilute to 400 µmol/L standard top solution by adding 0.2 ml stock solution into 0.8 ml distilled water. Perform 2-fold serial dilutions of the top standard solution using distilled water to make the standard curve. The concentration of standard curve could be 400/200/100/50/25/12.5/6.25 µmol/L.

Assay Buffer II: Briefly centrifuge prior to opening. Add 1.2 ml ethanol to dissolve before use. Keep in dark for immediate use or store at -20°C for 3-4 months after reconstitution.

Substrate: Add 19 ml Reaction Buffer to dissolve before use. Keep in dark for immediate use or store at -20°C for 1 week after reconstitution.

Note: Divide into small aliquots to avoid repeated freeze-thaw cycles.

V. SAMPLE PREPARATION

1. For cell and bacteria samples

Collect cell or bacteria into centrifuge tube, discard the supernatant after centrifugation, add 0.99 ml Assay Buffer I and 10 μ l Assay Buffer II for 5×10^6 cell or bacteria, sonicate (with power 20%, sonicate 3s, interval 10s, repeat 30 times); centrifuged at 8000g 4°C for 10 minutes, take the supernatant into a new centrifuge tube and keep it on ice for detection.

2. For tissue samples

Weigh out 0.1g tissue, homogenize with 0.99 ml Assay Buffer I and 10 μ l Assay Buffer II on ice, centrifuged at 8000g 4°C for 10 minutes, take the supernatant into a new centrifuge tube and keep it on ice for detection.

3. For liquid samples

Detect directly.

VI. ASSAY PROCEDURE

Warm all reagents to room temperature before use.

Add following reagents into the microplate:

Reagent*	Sample**	Standard	Blank
Substrate	190 μ l	--	--
Standard	--	200 μ l	--
Distilled water	--	--	200 μ l
Sample	10 μ l	--	--
Mix and wait for 2 minutes, measure at 340 nm and record the absorbance			

Note:

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

**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 samples into the reaction system; or increase the reaction time; if the enzyme activity is higher, please dilute the sample, or decrease the reaction time.

**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 of C₄H activity is defined as the enzyme products 1 μmol of NADPH per minute.

1. According to the slope of the standard curve

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

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

2.1 According to the protein concentration of sample

$$\text{Activity} = \frac{(C_{\text{Standard}} \times V_{\text{Standard}}) \times (\text{OD}_{\text{Sample}} - \text{OD}_{\text{Control}})}{(\text{OD}_{\text{Standard}} - \text{OD}_{\text{Blank}}) \times V_{\text{Sample}} \times C_{\text{Protein}} \times T} (\text{U/mg})$$

2.2 According to the quantity of cells or bacteria

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

2.3 According to the weight of sample

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

2.4 According to the volume of sample

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

Slope: the absorbance slope of standard curve

n: the dilution factor

C_{Protein}: the protein concentration of sample, mg/mL

W: the weight of total sample, g

N: the quantity of total cell or bacteria sample, 10⁴

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

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

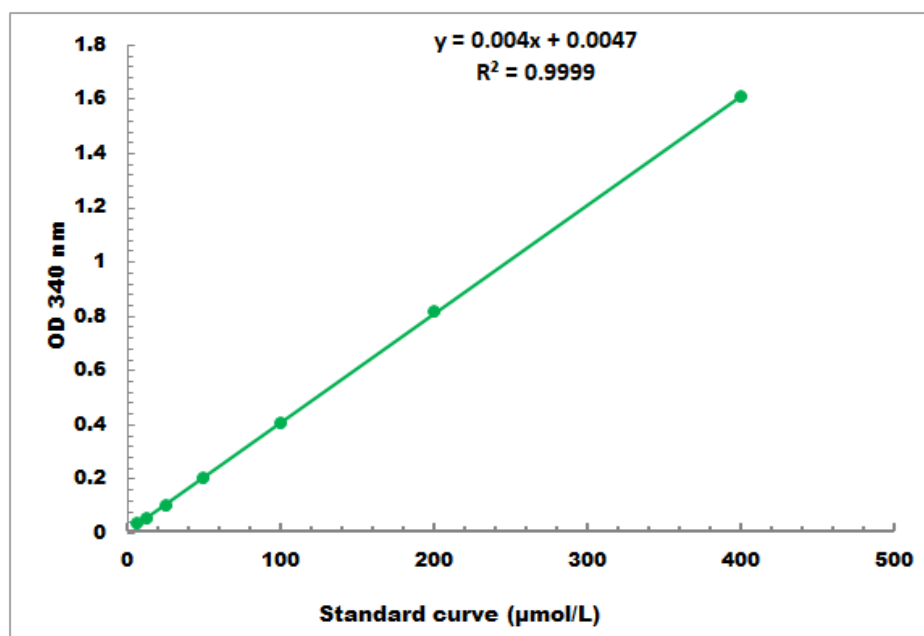
V_{Sample}: the volume of sample in assay procedure, mL

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