Cinnamate 4-hydroxylase Microplate Assay Kit User Manual

Catalog # FTA0176

(Version 1.2A)

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.

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 ρ-cinnamic acid.

Cinnamate 4-hydroxylase 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	30 ml x 4	4 °C
Substrate	Powder x 1	-20 °C
Reaction Buffer	20 ml x 1	4 °C
Standard	Powder x 1	-20 °C
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Note:

Substrate: add 19 ml Reaction Buffer 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, mix, 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. Ice
7. Centrifuge
8. Timer

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%, sonication 3s, intervation 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 1 ml Assay buffer 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.

V. ASSAY PROCEDURE

Warm all reagents to room temperature before use.

Add following reagents into the microplate:

Reagent	Sample	Standard	Blank
Substrate	190 µl	--	--
Reaction Buffer	--	--	--
Standard	--	200 µl	--
Distilled water	--	--	200 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.

VI. CALCULATION

Unit Definition: One unit of C4H activity is defined as the enzyme products 1 nmol of NADPH per minute.

1. According to the protein concentration of sample

$$C4H\ (U/mg) = (C_{Standard} \times V_{Standard}) \times (OD_{Sample(130S)} - OD_{Sample(10S)}) / (OD_{Standard} - OD_{Blank}) / (V_{Sample} \times C_{Protein}) / T$$

$$= 4000 \times (OD_{Sample(130S)} - OD_{Sample(10S)}) / (OD_{Standard} - OD_{Blank}) / C_{Protein}$$

2. According to the weight of sample

$$C4H\ (U/g) = (C_{Standard} \times V_{Standard}) \times (OD_{Sample(130S)} - OD_{Sample(10S)}) / (OD_{Standard} - OD_{Blank}) / (V_{Sample} \times W / V_{Assay}) / T$$

$$= 4000 \times (OD_{Sample(130S)} - OD_{Sample(10S)}) / (OD_{Standard} - OD_{Blank}) / W$$

3. According to the quantity of cell or bacteria

$$C4H\ (U/10^4) = (C_{Standard} \times V_{Standard}) \times (OD_{Sample(130S)} - OD_{Sample(10S)}) / (OD_{Standard} - OD_{Blank}) / (V_{Sample} \times N / V_{Assay}) / T$$

$$= 4000 \times (OD_{Sample(130S)} - OD_{Sample(10S)}) / (OD_{Standard} - OD_{Blank}) / N$$

4. According to the volume of sample

$$4CL\ (U/ml) = (C_{Standard} \times V_{Standard}) \times (OD_{Sample(130S)} - OD_{Sample(10S)}) / (OD_{Standard} - OD_{Blank}) / V_{Sample} / T$$

$$= 4000 \times (OD_{Sample(130S)} - OD_{Sample(10S)}) / (OD_{Standard} - OD_{Blank})$$

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

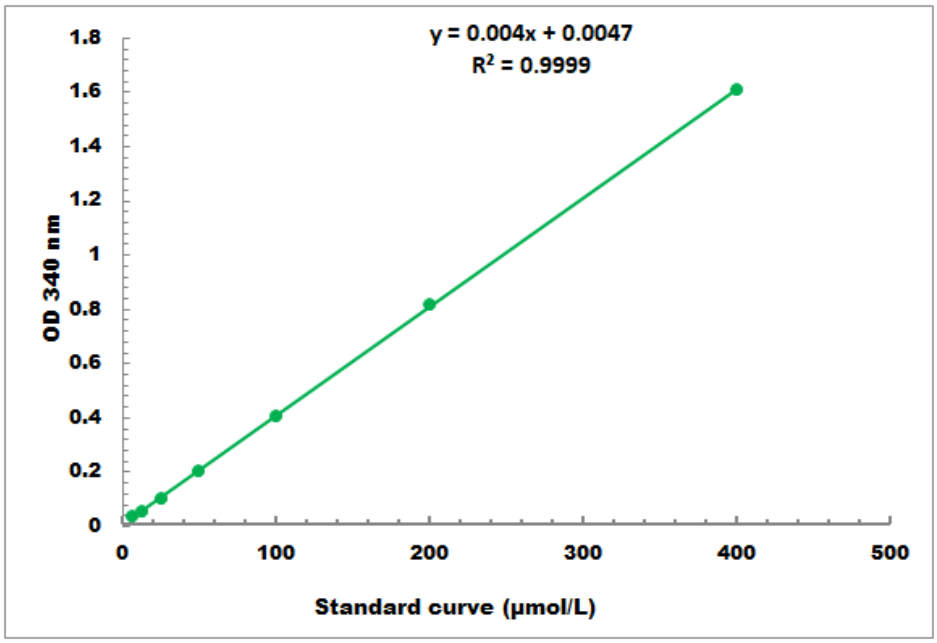
V_{Sample}: the volume of sample, 0.01 ml;

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