Cinnamate 4-hydroxylase Assay Kit

ARG83401 Cinnamate 4-hydroxylase Assay Kit can be used to measure Cinnamate 4-hydroxylase in tissue extracts, cell lysate, cell culture media and other biological fluids.

Catalog number: ARG83401

Package: 96 wells

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INTRODUCTION

Cinnamate 4-hydroxylase is key enzymes of the phenylpropanoid pathway, leading to the biosynthesis of several secondary metabolites.

PRINCIPLE OF THE ASSAY

The Cinnamate 4-hydroxylase Assay Kit can be measured Cinnamate 4-hydroxylase at a colorimetric readout at 340 nm.

MATERIALS PROVIDED & STORAGE INFORMATION

Component	Quantity	Storage
Microplate	1 X 96-well plate	
Standard	1 vial (lyophilized)	-20°C
Assay Buffer	4 X 30 ml (ready to use)	4°C
Reaction Buffer	20 ml	4°C
Substrate	1 vial (lyophilized)	-20°C

MATERIALS REQUIRED BUT NOT PROVIDED

- Microplate reader capable of measuring absorbance at 340 nm
- Pipettes and pipette tips
- Deionized or distilled water

TECHNICAL HINTS AND PRECAUTIONS

- Wear protective gloves, clothing, eye, and face protection especially while handling blood or body fluid samples.
- Standard and Substrate store at -20°C, all other component store at 4°C.
- Briefly spin down the reagents before use.
- It is highly recommended that the standards and samples be assayed in at least duplicates.
- Change pipette tips between the addition of different reagent or samples.

SAMPLE COLLECTION & STORAGE INFORMATION

The sample collection and storage conditions listed below are intended as general guidelines. Sample stability has not been evaluated.

Cell or bacteria lysate- Collect cell or bacteria into centrifuge tube, discard the supernatant after centrifugation, add 1 ml Assay buffer for 5×106 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.

Tissue lysate- 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.

Note: For other liquid sample, it can be assayed directly.

REAGENT PREPARATION

- **Standard:** Add **1 ml** of **distilled water** to dissolve before use; then add **200 µl standard** into **800 µl distilled water**. The concentration will be **400 nmol/ml**. Perform 2-fold serial dilution of the top standards to make the standard curve.
- **Substrate:** Reconstitute the Substrate with **19 ml** of **Reaction Buffer**. Allow the Substrate keep on bench for few minutes. Make sure the Substrate is dissolved completely and mixed thoroughly before use. Keep the reconstituted the Substrate on ice before use. Keep the reconstituted the Substrate on ice before use.
- **Sample**: If the measuring absorbance of samples is higher than the standard, dilute the samples with **Distilled water** before assay and assay again. For the calculation of the activity this dilution factor has to be taken into account.

ASSAY PROCEDURE

Standards and samples should be assayed in at least duplicates.

1. Sample wells: Add **190 µl Substrate** and **10 µl Samples** into Sample wells.
2. Standard wells: Add **200 µl Standard** into microplate.
3. Mix wells, read at 340 nm and record the OD of 10th second and 130th

Reagent	Sample	Standard	Blank
Substrate	190 µl	-	-
Standard	-	200 µl	-
Distilled water	-		200 µl
Sample	10 µl	-	-
Mix wells, read at 340 nm and record the OD of 10th second and 130th			

CALCULATION OF RESULTS

1. Unit Definition: One unit activity is defined as the enzyme that hydrolysis of one μmol arginine per minute.

2. Calculate the average absorbance values for each set of samples, standard, positive control, control and blank.

3. Calculation:

A. Definition:

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

$C_{Standard}$: the standard concentration, 400 nmol/ml;

W: the weight of sample, g;

V_{Sample}: the volume of reaction sample, 10 μl = 0.01 ml;

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

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

T: the reaction time, 2 minutes.

B. Formula:

a). According to the protein concentration of sample

Arginase activity (U/mg) =

$[(C_{Standard} \text{ X } V_{standard}) \text{ X } (OD_{Sample(130S)} - OD_{Sample(10S)})] / [(OD_{Standard} - OD_{Blank}) \text{ X } C_{Protein} \text{ X } V_{Sample} \text{ X } T]$

$=4000\text{X } (OD_{Sample(130S)} - OD_{Sample(10S)}) / [(OD_{Standard} - OD_{Blank}) \text{ X } C_{Protein}]$

b). According to the weight of sample

Arginase activity (U/g) =

$[(C_{Standard} \times V_{standard}) \times (OD_{Sample(130S)} - OD_{Sample(10S)})] / [(OD_{Standard} - OD_{Blank}) \times W \times V_{Sample} \times T]$

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

c). According to the quantity of cells or bacteria

Arginase activity ($U/10^4$) =

$[(C_{Standard} \times V_{standard}) \times (OD_{Sample(130S)} - OD_{Sample(10S)})] / [(OD_{Standard} - OD_{Blank}) \times N \times V_{Sample} \times T]$

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

d). According to the volume

Arginase activity (U/ml) =

$[(C_{Standard} \times V_{standard}) \times (OD_{Sample(130S)} - OD_{Sample(10S)})] / [(OD_{Standard} - OD_{Blank}) \times V_{Sample} \times T]$

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

4. Detection range:

The detection range is from 4 nmol/ml – 400 nmol/ml.

5. If the samples have been diluted, the calculated activity must be further converted by the appropriate dilution factor according to the sample preparation procedure as described above.

EXAMPLE OF TYPICAL RESULT

The following data is for demonstration only and cannot be used in place of data generations at the time of assay. Please note this data is for demonstration only and serial diluted standards are not necessary for this kit.