4-Coumarate CoA Ligase Assay Kit

ARG83400 4-Coumarate CoA Ligase Assay Kit can be used to measure 4-Coumarate CoA Ligase in tissue extracts, cell lysate, cell culture media and other biological fluids

Catalog number: ARG83400

Package: 96 wells

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INTRODUCTION

4-Coumarate:CoA ligase (4CL; EC 6.2.1.12) has a pivotal role in the biosynthesis of plant secondary compounds at the divergence point from general phenylpropanoid metabolism to several major branch pathways.

PRINCIPLE OF THE ASSAY

The ARG83400 4-Coumarate CoA Ligase Activity Assay Kit determined 4-Coumarate CoA Ligase. The increase in absorbance at 333 nm is directly proportional to the enzyme activity.

MATERIALS PROVIDED & STORAGE INFORMATION

Store the unopened kit at 2-8°C. Use the kit before expiration date.

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

MATERIALS REQUIRED BUT NOT PROVIDED

- Microplate reader capable of measuring absorbance at 540nm
- 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.
- Store Substrate at -20°C, other component 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.

For tissue- 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.

For cell and bacteria- 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.

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

REAGENT PREPARATION

- **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.

ASSAY PROCEDURE

Standards and samples should be assayed in at least duplicates.

1. Sample wells: Add **190 µl** per **Substrate** into each microplate.
2. Control wells: Add **190 µl** of **Reaction Buffer** into microplate.
3. Add **10 µl** of **Sample** into each wells.
4. Mix well. Incubate at **RT** for **5 min**. Read the OD at **333 nm**.

Summary of 4-Coumarate CoA Ligase Procedure

Reagent	Sample	Control
Substrate	190 µl	-
Reaction Buffer	-	190 µl
Sample	10 µl	10 µl
Incubate at **RT** for **5 min**. Read the OD at **333 nm**		

CALCULATION OF RESULTS

1. Unit Definition: One unit 4-Coumarate CoA Ligase activity is defined as the OD changed 0.01 per minute in the reaction system.

2. Calculate the average absorbance values for each set of samples and control.

3. Calculation:

A. Definition:

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

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

W: the weight of sample, g;

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

T: the reaction time, 5 minutes.

B. Formula:

a). According to the protein concentration of sample

4-Coumarate CoA Ligase activity (U/mg) =

$(OD_{Sample} - OD_{Control}) / [(C_{Protein} \times V_{Sample}) \times T \times 0.01]$

$= 2000 \times (OD_{Sample} - OD_{Control}) / C_{Protein}$

b). According to the weight of sample

4-Coumarate CoA Ligase activity (U/mg) =

$(OD_{Sample} - OD_{Control}) / [(W \times V_{Sample} / V_{assay}) \times T \times 0.01]$

$= 2000 \times (OD_{Sample} - OD_{Control}) / W$

c). According to the cell or bacteria

4-Coumarate CoA Ligase activity (U/10^4) =

$(OD_{Sample} - OD_{Control}) / [(N \times V_{Sample} / V_{assay}) \times T \times 0.01]$

$= 2000 \times (OD_{Sample} - OD_{Control}) / N$

d). According to the volume

4-Coumarate CoA Ligase activity (U/mg) =

$(OD_{Sample} - OD_{Control}) / (V_{Sample} \times T \times 0.01)$

$= 2000 \times (OD_{Sample} - OD_{Control})$