4-Coumarate CoA Ligase

Microplate Assay Kit

User Manual

Catalog # CAK1195

(Version 1.2A)

Detection and Quantification of 4-Coumarate CoA Ligase (4CL) 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

4-Coumarate CoA Ligase (EC 6.2.1.12) is a key enzyme in the lignin biosynthesis pathway, and it catalyzes a hydroxycinnamic acids and its derivatives to generate the corresponding thioester. Concurrently, 4 CL is also the third step in the metabolic pathway of phenylpropane, ligating the precursor of lignin and varied branch pathways, playing the critical regulating role in the lignin synthesis.

4-Coumarate CoA Ligase Microplate Assay Kit is a sensitive assay for determining 4-Coumarate CoA Ligase activity in various samples. The color intensity, measured at 333 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
Technical Manual	1 Manual	

Note:

Substrate: add 19 ml Reaction Buffer to dissolve before use.

III. MATERIALS REQUIRED BUT NOT PROVIDED

1. Microplate reader to read absorbance at 333 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%, 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 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	Control
Substrate	190 µl	--
Reaction Buffer	--	190 µl
Sample	10 µl	10 µl
Mix, incubate at room temperature for 5 minutes, record absorbance measured at 333nm.		

Note:

1) 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.

2) Reagents must be added step by step, can not be mixed and added together.

VI. CALCULATION

Unit Definition: One unit of 4CL activity is defined as the OD changed 0.01 per minute in the reaction system.

1. According to the protein concentration of sample

$$4CL\ (U/mg) = (OD_{Sample} - OD_{Control}) / (C_{Protein} \times V_{Sample}) / T / 0.01$$

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

2. According to the weight of sample

$$4CL\ (U/g) = (OD_{Sample} - OD_{Control}) / (W \times V_{Sample} / V_{Assay}) / T / 0.01$$

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

3. According to the quantity of cell or bacteria

$$4CL\ (U/10^4) = (OD_{Sample} - OD_{Control}) / (N \times V_{Sample} / V_{Assay}) / T / 0.01$$

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

4. According to the volume of sample

$$4CL\ (U/ml) = (OD_{Sample} - OD_{Control}) / V_{Sample} / T / 0.01$$

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

$C_{Protein}$: 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, 5 minutes.

VII. TECHNICAL SUPPORT

For troubleshooting, information or assistance, please go online to www.cohesionbio.com or contact us at techsupport@cohesionbio.com

VIII. NOTES