4-Coumarate CoA Ligase Activity

Colorimetric Microplate Assay Kit

User Manual

Catalog # CAK1195

(Version 1.3A)

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 catalyzesa hydroxycinnamic acids and its derivatives to generate the corresponding thioester. Concurrently,4 CL is also the third step in the metabolicpathway of phenylpropane,ligating the precursor of lignin and varied branch pathways,playing the critical regulating role in the ligninsynthesis.

4-Coumarate CoA LigaseActivity Colorimetric 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 I	30 ml x 4	4 °C
Assay Buffer II	Powder x 1	4 °C
Substrate	Powder x 1	-20 °C
Reaction Buffer	20 mlx 1	4 °C
Technical Manual	1 Manual	

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. REAGENT PREPARATION

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

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.

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 mlAssay Buffer Iand 10 µl 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 10minutes, take the supernatant into a new centrifuge tube and keep it on ice for detection.

2.For tissue samples

Weighout 0.1g tissue, homogenize with 0.99 mlAssay Buffer Iand 10 µl 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 reagentsto room temperature before use.

Add following reagents intothe 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 measure at 333nm.		

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: Oneunit of4CLactivity is defined as the OD changed 0.01 per minute in the reaction system.

1.1 According to the protein concentration of sample

$$\text{Activity} = \frac{V_{Total} \times (OD_{Sample} - OD_{Control})}{V_{Sample} \times C_{Protein} \times T \times 0.01} (U/mg)$$

1.2 According to the weight of sample

$$\text{Activity} = \frac{V_{Total} \times (OD_{Sample} - OD_{Control})}{W \times (V_{Sample} / V_{Assay}) \times T \times 0.01} (U/g)$$

1.3 According to the volume of sample

$$\text{Activity} = \frac{V_{Total} \times (OD_{Sample} - OD_{Control})}{V_{Sample} \times V / (V + V_{Assay}) \times T \times 0.01} (U/mL)$$

1.4 According to the quantity of cell or bacteria

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

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

W: the weight of sample, g

V: the volume of sample in sample preparation, ml

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

V_{Total}: thetotal volume of all reagents 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