4-Coumarate CoA Ligase Microplate Assay Kit

Catalog # AS0151

Detection and Quantification of 4-Coumarate CoA Ligase Activity in Tissue extracts, Cell lysate, Cell culture media, Other biological fluids Samples.

This instruction must be read in its entirety before using this product.

For research use only, Not for use in diagnostic procedures.

Contact information:

Tel:+1 (301) 446-2499 Fax:+1 (301) 446-2413

Email:techcn@signalwayantibody.com Web:www.sabbiotech.com

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 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 mlx 1	4 °C
Technical Manual	1 Manual	

Note:

Substrate: add 19 ml Reaction Bufferto dissolve before use.

III. MATERIALS REQUIRED BUT NOT PROVIDED

1. Microplate reader to read absorbance at 333 nm
2. Distilled water
3. 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 mlAssay 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 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 1mlAssay buffer on ice, centrifuged at 8000g 4°C for 10minutes, 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 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 measured at 333nm.		

VI. CALCULATION

Unit Definition: Oneunit of4CLactivity 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 ofsample

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

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

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

W: the weight of sample, g;

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

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.sabbiotech.cn or contact us at techcn@signalwayantibody.com

VIII. NOTES