Acetolactate Synthase

Microplate Assay Kit

User Manual

Catalog # CAK1174

(Version 1.2A)

Detection and Quantification of Acetolactate Synthase (ALS) activity in Tissue extracts, Cell lysate, Cell culture media, Other biological fluids Samples.

For research use only. Not for diagnostic or therapeutic procedures.

I. INTRODUCTION

The acetolactate synthase (EC 2.2.1.6), also known as acetohydroxy acid synthase, or AHAS, is a protein found in plants and micro-organisms. ALS catalyzes the first step in the synthesis of the branched-chain amino acids (valine, leucine, and isoleucine).

Acetolactate Synthase Microplate Assay Kit is a sensitive assay for determining acetolactate synthase activity in various samples. Acetolactate synthase activity is determined by the product of acetoin. The increase in absorbance at 525 nm is directly proportional to the enzyme activity.

II. KIT COMPONENTS

Component	Volume	Storage
96-Well Microplate	1 plate	
Assay Buffer	30 ml x 4	4 °C
Substrate	Powder x 1	4 °C
Stop Solution	1 ml x 1	4 °C
Dye Reagent	Powder x 1	4 °C
Dye Reagent Diluent	10 ml x 1	4 °C
Standard	Powder x 1	4 °C
Plate Adhesive Strips	3 Strips	
Technical Manual	1 Manual	

Note:

Substrate: add 5 ml Assay Buffer to dissolve before use.

Dye Reagent: add 10 ml Dye Reagent Diluent to dissolve before use.

Standard: add 1 ml distilled water to dissolve before use, then add 20 µl into 980 µl distilled water, mix; the concentration will be 1 mmol/L.

III. MATERIALS REQUIRED BUT NOT PROVIDED

1. Microplate reader to read absorbance at 525 nm
2. Distilled water
3. Pipettor, multi-channel pipettor
4. Pipette tips
5. Mortar
6. Centrifuge
7. Timer
8. Ice

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 0.1 g 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.

V. ASSAY PROCEDURE

Add following reagents into the microplate:

Reagent	Sample	Control	Standard	Blank
Sample	40 µl	--	--	--
Substrate	50 µl	50 µl	--	--
Distilled water	--	40 µl	50 µl	90 µl
Mix, put it in the oven, 37 °C for 1 hour.				
Stop Solution	10 µl	10 µl	10 µl	10 µl
Mix, put it in the oven, 60 °C for 15 minutes.				
Standard	--	--	40 µl	--
Dye Reagent	100 µl	100 µl	100 µl	100 µl
Mix, put it in the oven, 60 °C for 15 minutes, record absorbance measured at 525nm.				

Note:

1) Perform 2-fold serial dilutions of the top standards to make the standard curve.

2) 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.

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

VI. CALCULATION

Unit Definition: One unit of Acetolactate Synthase activity is defined as the enzyme generates 1 μmol acetoin per minute.

1. According to the protein concentration of sample

$$ALS\ (U/mg) = (C_{Standard} \times V_{Standard}) \times (OD_{Sample} - OD_{Control}) / (OD_{Standard} - OD_{Blank}) / V_{Sample} / C_{Protein} / T$$

$$= 0.0167 \times (OD_{Sample} - OD_{Control}) / (OD_{Standard} - OD_{Blank}) / C_{Protein}$$

2. According to the weight of sample

$$ALS\ (U/g) = (C_{Standard} \times V_{Standard}) \times (OD_{Sample} - OD_{Control}) / (OD_{Standard} - OD_{Blank}) / (V_{Sample} \times W / V_{Assay}) / T$$

$$= 0.0167 \times (OD_{Sample} - OD_{Control}) / (OD_{Standard} - OD_{Blank}) / W$$

3. According to the quantity of cell or bacteria

$$AST\ (U/10^4) = (C_{Standard} \times V_{Standard}) \times (OD_{Sample} - OD_{Control}) /(OD_{Standard} - OD_{Blank}) / (N \times V_{Sample} / V_{Assay})/T$$

$$= 0.0167 \times (OD_{Sample} - OD_{Control}) / (OD_{Standard} - OD_{Blank}) / N$$

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

W: the weight of sample, g;

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

$C_{Standard}$: the concentration of Standard, 1 mmol/L = 1 μmol/ml;

$V_{Standard}$: the total volume of standard, 0.04 ml;

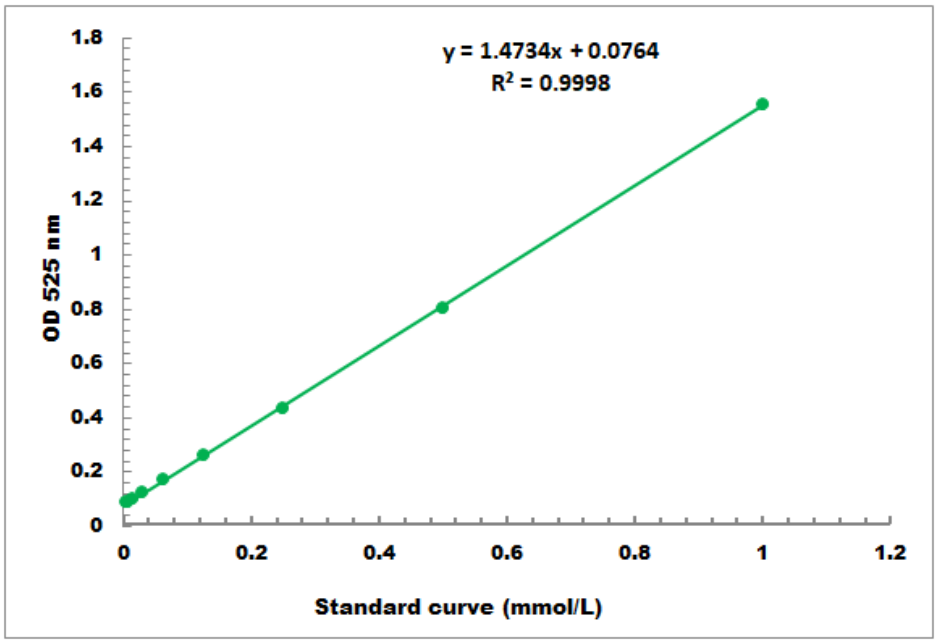
V_{Sample}: the volume of sample, 0.04 ml;

V_{Assay}: the volume of Assay buffer, 1 ml;

T: the reaction time, 60 minutes.

VII. TYPICAL DATA

The standard curve is for demonstration only. A standard curve must be run with each assay.



Detection Range: 0.01 mmol/L - 1 mmol/L

VIII. TECHNICAL SUPPORT

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

IX. NOTES