Isocitrate Lyase Activity

Colorimetric Microplate Assay Kit

User Manual

Catalog # CAK1072

(Version 1.3D)

Detection and Quantification of Isocitrate Lyase (ICL) 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

Isocitrate lyase (EC 4.1.3.1), or ICL, is an enzyme in the glyoxylate cycle that catalyzes the cleavage of isocitrate to succinate and glyoxylate. Together with malate synthase, it bypasses the two decarboxylation steps of the tricarboxylic acid cycle (TCA cycle) and is used by bacteria, fungi, and plants.

The assay is initiated with the enzymatic decomposition of the Isocitric acid by Isocitrate lyase. The enzyme catalysed reaction product NADH can be measured at a colorimetric readout at 340 nm.

II. KIT COMPONENTS

Component	Volume	Storage
96-Well Microplate	1 plate	
Assay Buffer	30 ml x 4	4 °C
Enzyme	Powder x 1	-20 °C
Substrate	Powder x 1	-20 °C
Standard	Powder x 1	-20 °C
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Note:

Enzyme: add 1 ml distilled water to dissolve before use.

Substrate: add 18 ml distilled water to dissolve before use.

Standard: add 1 ml distilled water to dissolve before use; then add 0.2 ml into 0.8 ml distilled water, the concentration will be 400 µmol/L.

III. MATERIALS REQUIRED BUT NOT PROVIDED

1. Microplate reader to read absorbance at 340 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 16000g 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 16000g 4 °C for 10 minutes, take the supernatant into a new centrifuge tube and keep it on ice for detection.

3. For other biological fluids samples

Detect directly.

V. ASSAY PROCEDURE

Add following reagents into the microplate:

Reagent	Sample	Standard	Blank
Sample	10 µl	--	--
Enzyme	10 µl	--	--
Substrate	180 µl	--	--
Standard	--	200 µl	--
Distilled water	--	--	200 µl
Mix, incubate at 37 °C and record the absorbance of 10th second and 130th second.			

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 Isocitrate Lyase activity is defined as the enzyme decomposes 1 μmol of the NADH per minute.

1. According to the protein concentration of sample

$$ICL\ (U/mg) = (C_{Standard} \times V_{Standard}) \times (OD_{Sample(10S)} - OD_{Sample(130S)}) / (OD_{Standard} - OD_{Blank}) / (V_{Sample} \times C_{Protein}) / T$$

$$= 4 \times (OD_{Sample(10S)} - OD_{Sample(130S)}) / (OD_{Standard} - OD_{Blank}) / C_{Protein}$$

2. According to the weight of sample

$$ICL\ (U/g) = (C_{Standard} \times V_{Standard}) \times (OD_{Sample(10S)} - OD_{Sample(130S)}) / (OD_{Standard} - OD_{Blank}) / (W \times V_{Sample} / V_{Assay}) / T$$

$$= 4 \times (OD_{Sample(10S)} - OD_{Sample(130S)}) / (OD_{Standard} - OD_{Blank}) / W$$

3. According to the quantity of cells or bacteria

$$ICL\ (U/10^4) = (C_{Standard} \times V_{Standard}) \times (OD_{Sample(10S)} - OD_{Sample(130S)}) / (OD_{Standard} - OD_{Blank}) / (N \times V_{Sample} / V_{Assay})/T$$

$$= 4 \times (OD_{Sample(10S)} - OD_{Sample(130S)}) / (OD_{Standard} - OD_{Blank}) / N$$

4. According to the volume of sample

$$ICL\ (U/ml) = (C_{Standard} \times V_{Standard}) \times (OD_{Sample(10S)} - OD_{Sample(130S)}) / (OD_{Standard} - OD_{Blank}) / V_{Sample}/ T$$

$$= 4 \times (OD_{Sample(10S)} - OD_{Sample(130S)}) / (OD_{Standard} - OD_{Blank})$$

$C_{Standard}$: the standard concentration, 400 μmol/L = 0.4 μmol/ml;

$V_{Standard}$: the volume of standard, 200 μl = 0.2 ml;

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

W: the weight of sample, g;

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

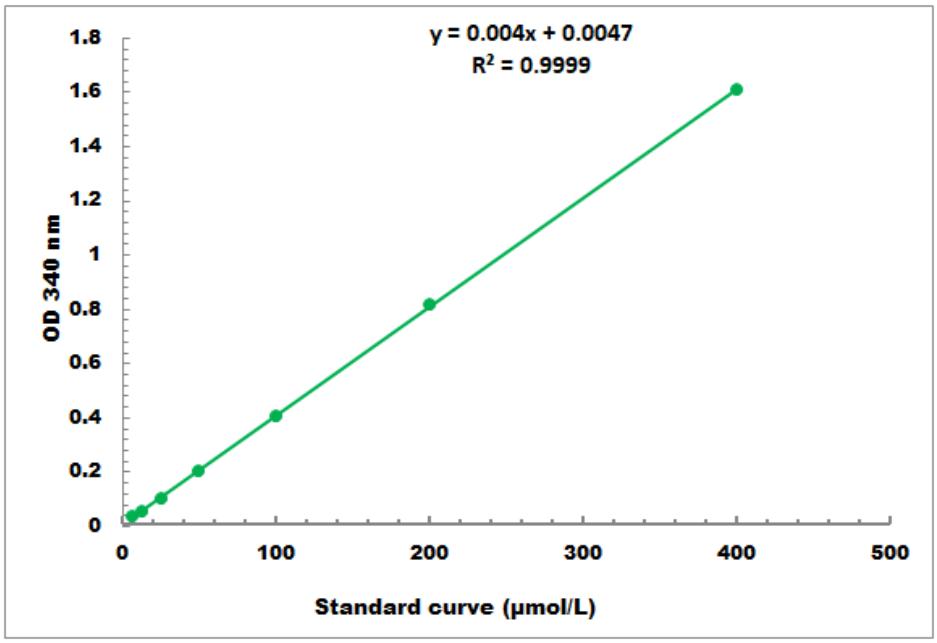
V_{Sample}: the volume of sample, 0.01 ml;

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

T: the reaction time, 2 minutes.

VII. TYPICAL DATA

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



Detection Range: 4 μmol/L - 400 μmol/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