Isocitrate Lyase Activity

Colorimetric Microplate Assay Kit

User Manual

Catalog # CAK1072

(Version 1.5E)

Detection and Quantification ofIsocitrate Lyase(ICL) Activityin Tissue extracts, Cell lysate, Cell culture media and Other biological fluidsSamples.

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.

Isocitrate Lyase ActivityColorimetric Microplate Assay Kit provides a simple and direct procedure for measuring isocitrate lyaseactivity in a variety of samples.The assay is initiated with the enzymatic decomposition of the isocitric acid byIsocitrate lyase. The enzyme catalysed reaction product NADHcan be measured at a colorimetric readout at 450 nm.

II.KIT COMPONENTS

Component	Volume	Storage
96-WellMicroplate	1 plate	
Assay Buffer	30 mlx 4	4 °C
Enzyme	Powder x 1	-20 °C
Substrate	Powder x 1	-20 °C
Dye ReagentA	Powder x 1	4 °C
Dye Reagent B	1 ml x 1	4 °C
Standard	Powder x 1	-20 °C
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III. MATERIALS REQUIRED BUT NOT PROVIDED

1. Microplate reader to read absorbance at 450 nm
2. Distilled water
3. Pipettor, multi-channel pipettor
4. Pipette tips
5. Mortar
6. Centrifuge
7. Timer
8. Ice

IV. REAGENTPREPARATION

Standard:Briefly centrifuge prior to opening.Dissolvein1 ml distilled waterto generate 2 mmol/L of standardstock solution, store at 4 °C for 1 day or -20°C for 3daysafter reconstitution.Then dilute to400 µmol/Lstandard top solutionby adding0.2 ml stock solution into 0.8 mldistilled water**.**Perform 2-fold serial dilutions of the top standardsolution using distilled waterto make the standard curve.The concentration of standard curve could be400/200/100/50/25/12.5/6.25µmol/L.

Enzyme: Briefly centrifuge prior to opening. Add 1 ml distilled waterto dissolve before use.Store at -80°C for 1 month after reconstitution.

Substrate: Add 12ml distilled water to dissolve before use. Keep in dark for immediate use or store at -20°C for 1 week after reconstitution.

Dye Reagent A: Briefly centrifuge prior to opening. Add 5ml distilled water to dissolve before use, mix, store at 4°C for 1 month 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 1 mlAssay 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

Weighout 0.1 g tissue, homogenize with 1 mlAssay 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 intothe microplate:

Reagent*	Sample**	Control	Standard	Blank
Substrate	120 µl	120 µl	--	--
Sample	10 µl	--	--	--
Distilled water	--	10 µl	40 µl	140 µl
Enzyme	10 µl	10 µl	--	--
Mix andincubate at 30℃ for 5 minutes.				
Standard	--	--	100µl	--
Dye Reagent A	50 µl	50 µl	50 µl	50 µl
Dye Reagent B	10 µl	10 µl	10 µl	10 µl
Mix and incubate at 37°C for 2 minutes, recordthe absorbance.				

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.

VI. CALCULATION

Unit Definition: One unit ofIsocitrate Lyase activity is defined asthe enzymedecomposes 1μmol ofthe NADH per minute.

1. According to the slope of the standard curve

$$\text{Activity} = \frac{(OD_{Control}-OD_{Sample}) - \text{Intercept}}{\text{Slope}\times T} \times \frac{V_{Standard}}{V_{Sample}} \times n(U/mL)$$

2. According to one point of the standard OD value and concentration

2.1 According to the protein concentration of sample

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

2.2According to the quantity of cells or bacteria

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

2.3 According to the weight of sample

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

2.4 According to the volume of sample

$$\text{Activity}=\frac{(C_{Standard}\times V_{Standard}) \times (OD_{Control}-OD_{Sample})}{(OD_{Standard}-OD_{Blank}) \times V_{Sample}\times T}(U/mL)$$

Slope: the absorbance slope of standard curve

n: the dilution factor

$C_{Protein}$: the protein concentration of sample, mg/mL

W: the weight of total sample, g

N: the quantity of total cell or bacteria sample, 10^4

$C_{Standard}$: the concentration of standard, μmol/L

$V_{Standard}$: the volume of standard in assay procedure, mL

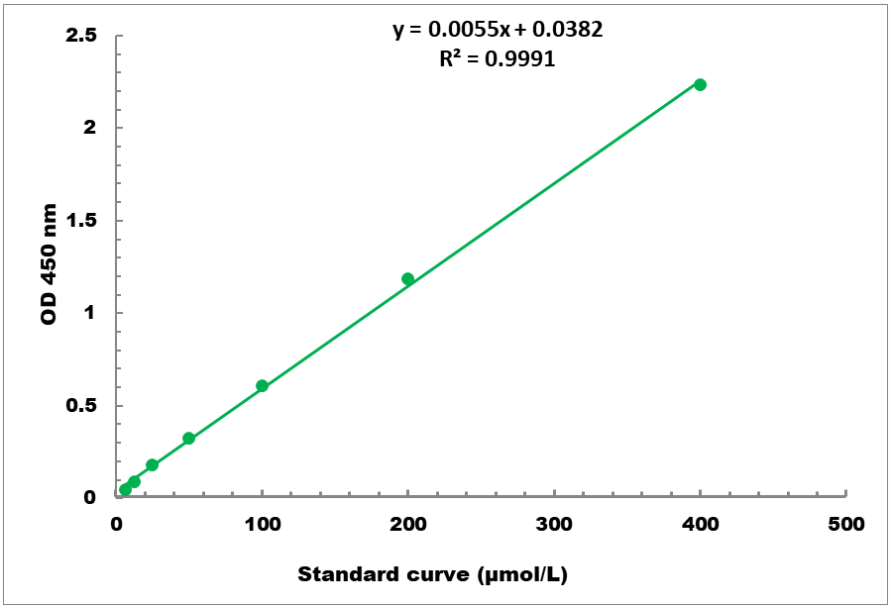
V_{Sample}: the volume of sample in assay procedure, mL

V_{Assay}: the volume of Assay Buffer in sample preparation, mL

T: the reaction time, minute

VIII. TYPICAL DATA

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



Detection Range: 4μmol/L - 400 μmol/L