Isocitrate Microplate Assay Kit

User Manual

Catalog # CAK1202

(Version 1.4C)

Detection and Quantification of Isocitrate Content in Serum, Plasma, Tissue extracts, Cell lysate, Cell culture media and Other biological fluids Samples.

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

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I. INTRODUCTION

Isocitrate is a substrate in the citric acid (TCA) cycle. Isocitrate is formed by the isomerization of citrate catalyzed by the enzyme aconitase. Isocitrate is oxidized by isocitrate dehydrogenase producing α-ketoglutarate and generating NADPH. Isocitrate is commonly found in many fruits and vegetables and their processed products. Industrially, isocitrate is used as a marker to identify the quality and purity of fruit juices.

Isocitrate Microplate Assay Kit provides a simple and direct procedure for measuring isocitrate content in a variety of samples. The NADPH converts the dye to an intense violet color with an absorption maximum at 450 nm. The increase in absorbance at 450 nm is directly proportional to the isocitrate concentration.

II. KIT COMPONENTS

Component	Volume	Storage
96-Well Microplate	1 plate	
Assay Buffer	30 ml x 4	4 °C
Reaction Buffer	10 ml x 1	4 °C
Coenzyme	Powder x 1	-20 °C
Enzyme	100 µl x 1	4 °C
Dye Reagent A	Powder x 1	4 °C
Dye Reagent B	1 ml x 1	4 °C
Standard	Powder x 1	-20 °C
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Note:

Coenzyme: add 1 ml Reaction Buffer to dissolve before use.

Enzyme: add 1 ml Reaction Buffer to dissolve before use.

Dye Reagent A: add 9 ml distilled water to dissolve before use, mix, store at 4°C.

Standard: add 1 ml distilled water to dissolve before use; then add 0.5 ml into 0.5 ml distilled water, the concentration will be 5 mmol/L.

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. 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.

3. For liquid samples

Detect directly.

V. ASSAY PROCEDURE

Warm all regents to room temperature before use.

Add following reagents into the microplate:

Reagent	Sample	Standard	Blank
Reaction Buffer	60 µl	60 µl	60 µl
Sample	20 µl	--	--
Standard	--	20 µl	--
Distilled water	--	--	20 µl
Coenzyme	10 µl	10 µl	10 µl
Enzyme	10 µl	10 µl	10 µl
Incubate at room temperature for 30 minutes.			
Dye Reagent A	90 µl	90 µl	90 µl
Dye Reagent B	10 µl	10 µl	10 µl
Mix, wait for 15 minutes, measured at 450 nm and record the absorbance.			

Note:

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

2) The concentrations can vary over a wide range depending on the different samples. For unknown samples, we recommend doing a pilot experiment & testing several doses to ensure the readings are within the standard curve range.

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

VI. CALCULATION

1. According to the protein concentration of sample

$$\text{Isocitrate } (\mu\text{mol/mg}) = (C_{Standard} \times V_{Standard}) \times (OD_{Sample} - OD_{Blank}) / (OD_{Standard} - OD_{Blank}) / (V_{Sample} \times C_{Protein})$$

$$= 2.5 \times (OD_{Sample} - OD_{Blank}) / (OD_{Standard} - OD_{Blank}) / C_{Protein}$$

2. According to the weight of sample

$$\text{Isocitrate } (\mu\text{mol/g}) = (C_{Standard} \times V_{Standard}) \times (OD_{Sample} - OD_{Blank}) / (OD_{Standard} - OD_{Blank}) / (V_{Sample} \times W / V_{Assay})$$

$$= 2.5 \times (OD_{Sample} - OD_{Blank}) / (OD_{Standard} - OD_{Blank}) / W$$

3. According to the volume of serum or plasma

$$\text{Isocitrate } (\mu\text{mol/ml}) = (C_{Standard} \times V_{Standard}) \times (OD_{Sample} - OD_{Blank}) / (OD_{Standard} - OD_{Blank}) / V_{Sample}$$

$$= 2.5 \times (OD_{Sample} - OD_{Blank}) / (OD_{Standard} - OD_{Blank})$$

$C_{Standard}$: the standard concentration, 5 mmol/L = 5 μmol/ml;

$V_{Standard}$: the volume of standard, 20 μl = 0.02 ml;

V_{Sample}: the volume of sample, 20 μl = 0.02 ml;

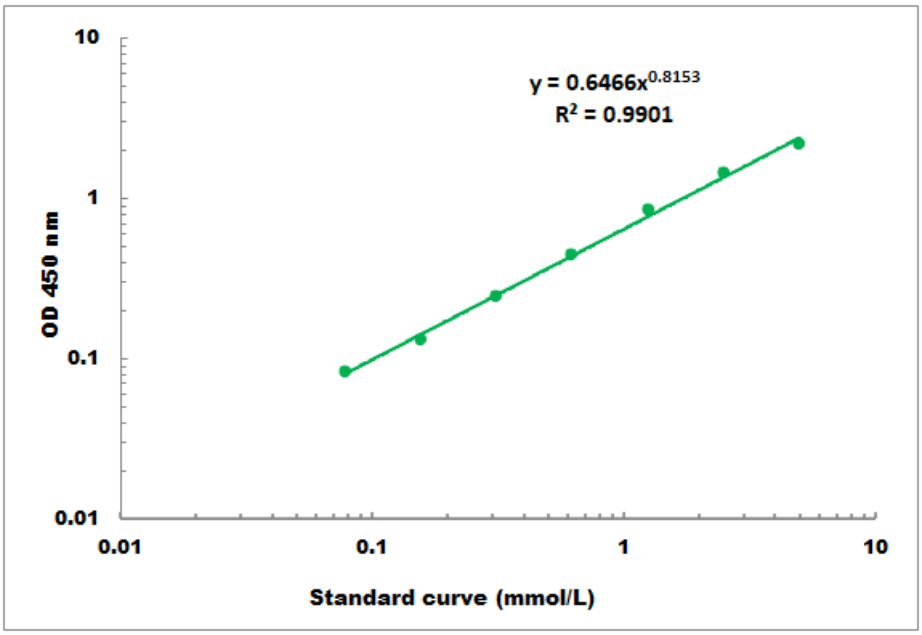
$C_{Protein}$: the protein concentration, mg/ml;

W: the weight of sample, g;

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

VII. TYPICAL DATA

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



Detection Range: 0.1 mmol/L - 5 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