



Isocitrate

Colorimetric Microplate Assay Kit

User Manual

Catalog # CAK1202

(Version 1.5C)

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.

I. INTRODUCTION.....	2
II. KIT COMPONENTS.....	3
III. MATERIALS REQUIRED BUT NOT PROVIDED.....	4
IV. REAGENT PREPARATION.....	5
V. SAMPLE PREPARATION.....	6
VI. ASSAY PROCEDURE.....	7
VII. CALCULATION.....	8
VIII. TYPICAL DATA.....	9

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 Colorimetric 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	30ml x 4	4 °C
Reaction Buffer	10 ml x 1	4 °C
Coenzyme	Powder x 1	-20 °C
Enzyme	100µl x 1	4 °C
Dye Reagent A	Powder x 1	4 °C, keep in dark
Dye Reagent B	1 ml x 1	4 °C, keep in dark
Standard	Powder x 1	-20 °C
Technical Manual	1 Manual	

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. REAGENT PREPARATION

Standard: Briefly centrifuge prior to opening. Dissolve in 1 ml distilled water to generate 10 mmol/L of standard stock solution, store at 4 °C for 2-3 weeks or -20°C for 4-5 months after reconstitution. Then dilute to 5 mmol/L standard top solution by adding 0.5 ml stock solution into 0.5 ml distilled water. Perform 2-fold serial dilutions of the top standard solution using distilled water to make the standard curve. The concentration of standard curve could be 5/2.5/1.25/0.625/0.312/0.156/0.078 mmol/L.

Coenzyme: Briefly centrifuge prior to opening. Add 1 ml Reaction Buffer to dissolve before use. Store at 4 °C for 2-3 weeks or -20°C for 4-5 months after reconstitution.

Enzyme: Briefly centrifuge prior to opening. Add 1 ml Reaction Buffer to dissolve before use. Store at -20°C for 1-2 months after reconstitution.

Dye Reagent A: Add 9 ml distilled water to dissolve before use, mix, store at 4°C. Keep in dark and store at 4°C for 1 week or -20°C for 1 month.

V. 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.

VI. ASSAY PROCEDURE

Warm all reagents to room temperature before use.

Add following reagents into the microplate:

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

Note:

*Reagents must be added sequentially and should not be premixed prior to addition.

**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.

**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.

VII. CALCULATION

1. Calculate the sample concentration in ASSAY PROCEDURE according to the slope of the standard curve

$$C = \frac{(OD_{\text{Sample}} - OD_{\text{Blank}}) - \text{Intercept}}{\text{Slope}} \times \frac{V_{\text{Standard}}}{V_{\text{Sample}}} \times n (\text{mmol/L})$$

Calculate the initial concentration according to sample preparation procedure.

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

2.1 According to the quantity of cells or bacteria

$$C = \frac{(C_{\text{Standard}} \times V_{\text{Standard}}) \times (OD_{\text{Sample}} - OD_{\text{Blank}})}{(OD_{\text{Standard}} - OD_{\text{Blank}}) \times N \times (V_{\text{Sample}} / V_{\text{Assay}})} (\text{mmol}/10^4)$$

2.2 According to the weight of sample

$$C = \frac{(C_{\text{Standard}} \times V_{\text{Standard}}) \times (OD_{\text{Sample}} - OD_{\text{Blank}})}{(OD_{\text{Standard}} - OD_{\text{Blank}}) \times W \times (V_{\text{Sample}} / V_{\text{Assay}})} (\text{mmol/g})$$

2.3 According to the volume of sample

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

Slope: the absorbance slope of standard curve

n: the dilution factor

C_{Standard} : the standard concentration, mmol/L = $\mu\text{mol/ml}$

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

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

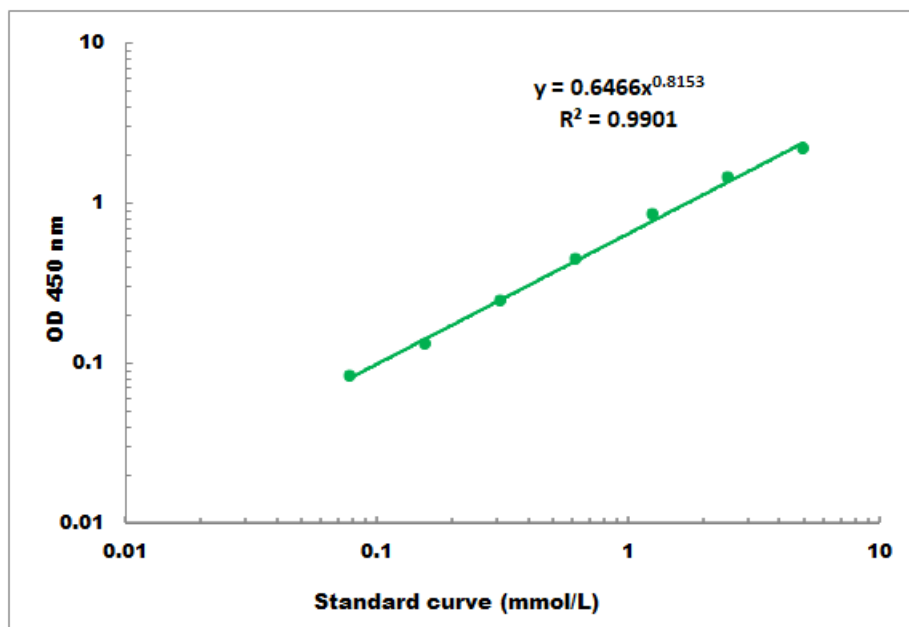
V_{Assay} : the volume of Assay Buffer, μl

W: the weight of sample, g

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

VIII. TYPICAL DATA

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



Detection Range: 0.05mmol/L - 5mmol/L