Citrate Microplate Assay Kit

User Manual

Catalog # FTA0068

(Version 1.2D)

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

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

I. INTRODUCTION

Citrate is a key tricarboxylic acid (TCA) cycle intermediate formed by the addition of oxaloacetate to the acetyl group of acetyl-CoA. Citrate is transported out of the mitochondria via the citrate-malate shuttle and converted back to acetyl-CoA for fatty acid synthesis. Citrate is an allosteric modulator of both fatty acid synthesis via its actions on acetyl-CoA carboxylase and of glycolysis via its actions on phosphofructokinase. Citrate metabolism and disposition can vary widely due to sex, age, and a variety of other factors including disease states. Cellular citrate levels are decreased in prostrate cancer cells and citrate levels may be a marker of prostrate cancer growth rate.

Citrate Microplate Assay Kit is a sensitive assay for determining citrate concentration in various samples. Citrate breaks down into keto acid by citrate lyase. The color intensity, measured at 340 nm, is proportionate to the citrate concentration in the sample.

II. KIT COMPONENTS

Component	Volume	Storage
96-Well Microplate	1 plate	
Assay Buffer	30 ml x 4	4 °C
Reaction Buffer	15 ml x 1	4 °C
Enzyme	Powder x 1	-20 °C
Dye Reagent	Powder x 1	4 °C, keep in dark
Standard	Powder x 1	4 °C
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Note:

Enzyme: add 2 ml Assay buffer to dissolve before use.

Dye Reagent: add 2 ml distilled water to dissolve before use.

Standard: add 1 ml distilled water to dissolve before use; then add 50 µl into 950 µl distilled water, the concentration will be 5 mmol/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

IV. SAMPLE PREPARATION

1. For urine, serum or plasma samples

Detect directly, or dilute with Assay Buffer.

2. For tissue samples

Weigh out 0.1 g tissue, homogenize with 1 ml Assay buffer on ice, centrifuged at 10000g 4 °C for 10 minutes, take the supernatant into a new centrifuge tube for detection.

V. ASSAY PROCEDURE

Add following reagents in the microplate:

Reagent	Sample	Standard	Blank
Sample	10 µl	--	--
Standard	--	10 µl	--
Distilled water	--	--	10 µl
Reaction Buffer	150 µl	150 µl	150 µl
Enzyme	20 µl	20 µl	20 µl
Dye Reagent	20 µl	20 µl	20 µl
Mix, incubate at room temperature for 30 minutes, measured at 340 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.

VI. CALCULATION

1. According to the volume of sample

$$\text{Citrate (μmol/ml)} = (C_{Standard} \times V_{Sample}) \times (OD_{Sample} - OD_{Blank}) / (OD_{Standard} - OD_{Blank}) / V_{Standard}$$

$$= 5 \times (OD_{Sample} - OD_{Blank}) / (OD_{Standard} - OD_{Blank})$$

2. According to the weight of sample

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

$$= 5 \times (OD_{Sample} - OD_{Blank}) / (OD_{Standard} - OD_{Blank}) / W$$

3. According to the protein concentration of sample

$$\text{Citrate (μmol/mg)} = (C_{Standard} \times V_{Sample}) \times (OD_{Sample} - OD_{Blank}) / (OD_{Standard} - OD_{Blank}) / (V_{Sample} \times C_{Protein})$$

$$= 5 \times (OD_{Sample} - OD_{Blank}) / (OD_{Standard} - OD_{Blank}) / C_{Protein}$$

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

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

W: the weight of sample, g;

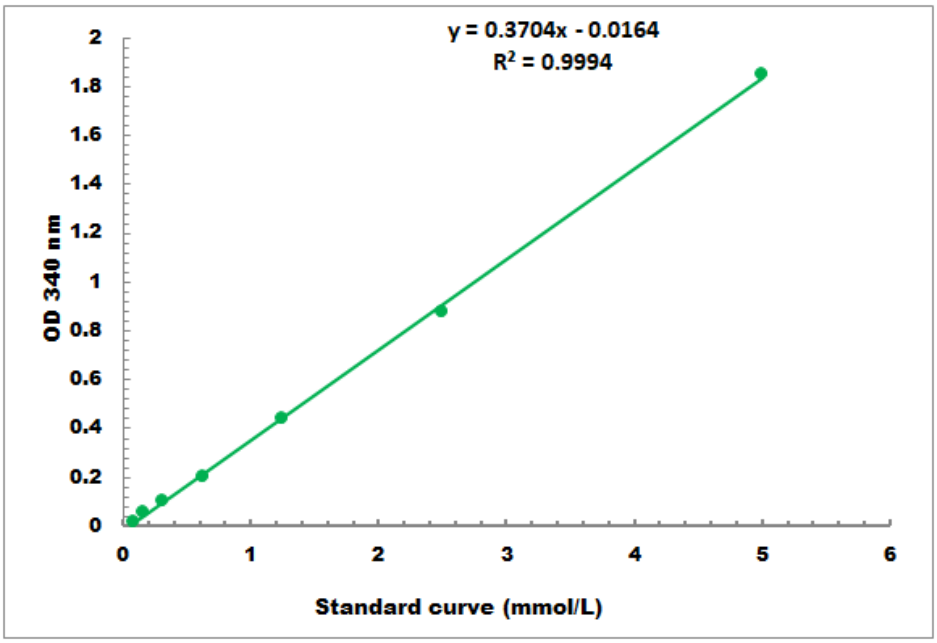
V_{Sample}: the volume of sample, 0.01 ml;

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

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: 0.05 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