Citrate Microplate Assay Kit

Cat #: orb390775 (manual)

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

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

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 prostate cancer cells and citrate levels may be a marker of prostate cancer growth rate.

Citrate Microplate Assay Kit is a sensitive assay for determining citrate concentration in various samples. Citrate breaks down into malate by citrate lyase. The amount of NAD+ formed in the above reaction pathway is stoichiometric with the amount of citric acid. It is NADH consumption which is measured by the decrease in absorbance at 450 nm.

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
Enzyme Mix	Powder x 1	-20 °C
Coenzyme	Powder 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	4 °C
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Note:

Enzyme Mix: add 1 ml Reaction Buffer to dissolve before use. Store at -80°C.

Coenzyme: add 1 ml Reaction Buffer to dissolve before use. Store at -80°C.

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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 200 µl into 800 µl distilled water, the concentration will be 10 mmol/L.

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

SAMPLE PREPARATION

1. For liquid 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.

ASSAY PROCEDURE

Add following reagents in the microplate:

Reagent	Sample	Standard	Control
Reaction Buffer	70 µl	70 µl	70 µl
Sample	10 µl	--	--
Standard	--	10 µl	--
Distilled water	--	--	10 µl
Enzyme Mix	10 µl	10 µl	10 µl
Coenzyme	10 µl	10 µl	10 µl
Dye Reagent A	90 µl	90 µl	90 µl
Dye Reagent B	10 µl	10 µl	10 µl
Mix, keep at RT for 1 minute, 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, cannot be mixed and added together.

CALCULATION

1. According to the volume of sample

Citrate (μmol/ml) = ($C_{Standard}$ × V_{Sample}) × ($OD_{Control}$ - OD_{Sample}) / ($OD_{Control}$ - $OD_{Standard}$)/ $V_{Standard}$

= 10 × ($OD_{Control}$ - OD_{Sample}) / ($OD_{Control}$ - $OD_{Standard}$)

2. According to the weight of sample

Citrate (μmol/g) = ($C_{Standard}$ × V_{Sample}) × ($OD_{Control}$ - OD_{Sample}) / ($OD_{Control}$ - $OD_{Standard}$) / (W × V_{Sample} / V_{Assay})

= 10 × ($OD_{Control}$ - OD_{Sample}) / ($OD_{Control}$ - $OD_{Standard}$) / W

3. According to the protein concentration of sample

Citrate (μmol/mg) = ($C_{Standard}$ × V_{Sample}) × ($OD_{Control}$ - OD_{Sample}) / ($OD_{Control}$ - $OD_{Standard}$) / (V_{Sample} × $C_{Protein}$)

= 10 × ($OD_{Control}$ - OD_{Sample}) / ($OD_{Control}$ - $OD_{Standard}$) / $C_{Protein}$

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

$C_{Standard}$: the standard concentration, 10 mmol/L = 10 μmol/ml

W: the weight of sample, g

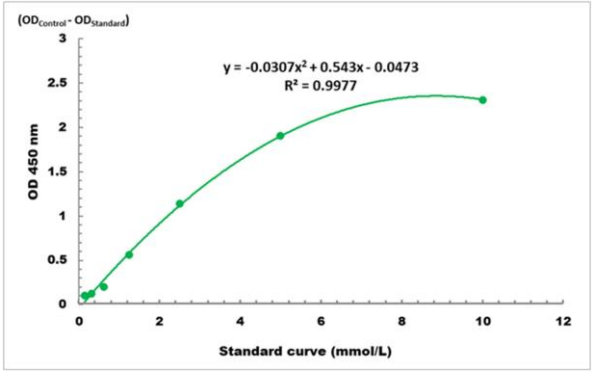
V_{Sample}: the volume of sample, 0.01 ml

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

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

TYPICAL DATA

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



Detection Range: 0.1 mmol/L - 10 mmol/L