

## Instructions for Use

Version: 1.0.1

Revision date: 3-Aug-22

# Citrate Assay Kit

**Catalog No.:** abx298835

**Size:** 100 Assays

**Storage:** Store all components in the dark at 4°C.

**Application:** For quantitative detection of Citrate concentrations in serum, plasma, tissue homogenates, cell lysates, cell culture supernatants, urine, and other biological fluids.

**Detection Range:** 0.05 mmol/L – 5 mmol/L

**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.

Abbexa's Citrate Assay Kit is a quick, convenient, and sensitive method for measuring and calculating Citrate concentrations. Citrate is broken down into keto acid by citrate lyase, which can be measured spectrophotometrically at 340 nm. The intensity of the color is proportional to the concentration of Citrate, which can then be calculated.

### Kit components

1. 96 well microplate
2. Assay Buffer: 4 x 30 ml
3. Reaction Buffer: 1 x 15 ml
4. Enzyme: 1 vial
5. Dye Reagent: 1 vial
6. Standard: 1 vial
7. Plate sealer: 3

### Materials Required But Not Provided

1. Microplate reader (340 nm)
2. Centrifuge and microcentrifuge tubes
3. High-precision pipette and sterile pipette tips
4. Distilled water
5. Timer
6. Ice
7. Sonicator
8. Mortar
9. Water bath

## Protocol

### A. Preparation of Sample and Reagents

#### 1. Reagents

- **Enzyme**

Add 2 ml of Assay Buffer into the Enzyme vial and mix thoroughly to prepare Enzyme Solution

- **Dye Reagent**

Add 2 ml of distilled water into the Dye Reagent vial and mix thoroughly to prepare the Dye Reagent Solution. Ensure that the Dye Reagent has completely dissolved prior to use.

- **Standard Solution**

Add 1 ml of distilled water into the Standard vial and mix thoroughly. Ensure that the Standard has completely dissolved. Take 50 µl of this solution and add 950 µl of distilled water to prepare the Standard Solution (concentration 5 mmol/L).

#### 2. Sample

- **Tissue samples**

Homogenize 0.1 g of sample in 1 ml of Assay Buffer on ice. Centrifuge at 10,000 x g at 4°C for 10 minutes. Transfer the supernatant to a new pre-cooled tube, then analyze immediately.

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- **Urine, serum and plasma samples**

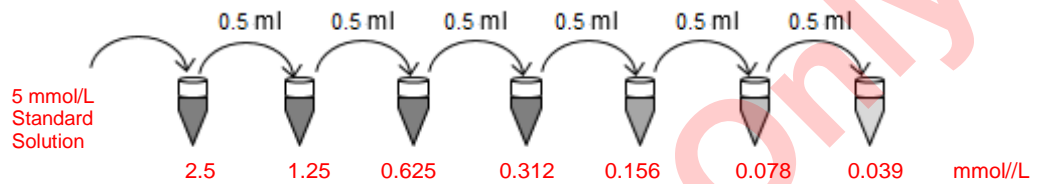
Urine, serum and plasma samples can be used directly.

### B. Assay Procedure

Warm all reagents to 37°C prior to use.

If the expected concentration is unknown, a preliminary experiment is recommended to determine if sample dilutions or adjustments to the reaction time are required to bring the measured concentration within the detection range of the kit.

1. Label 7 tubes with 2.5 mmol/L, 1.25 mmol/L, 0.625 mmol/L, 0.312 mmol/L, 0.156 mmol/L, 0.078 mmol/L, and 0.039 mmol/L. Aliquot 0.5 ml of distilled water into each tube. Add 0.5 ml of 5 mmol/L Standard Solution to the 1<sup>st</sup> tube and mix thoroughly. Transfer 0.5 ml from the 1<sup>st</sup> tube to the 2<sup>nd</sup> tube and mix thoroughly, and so on.



2. Set the sample, standard and blank wells on the 96 well microplate and record their positions. We recommend setting up each standard and sample in duplicate.
3. Add 10 µl of sample to the sample wells.
4. Add 10 µl of prepared standards to the standard wells.
5. Add 10 µl of distilled water to the blank wells.
6. Add 150 µl of Reaction Buffer to all wells.
7. Add 20 µl of Enzyme and Dye Reagent Solution to all wells.
8. Tap the plate gently to mix. Allow to stand for 30 minutes. Read and record absorbance at 340 nm.

### C. Calculations

Citrate concentration per mg of protein:

$$\text{Citrate } (\mu\text{mol/mg}) = \frac{C_{\text{Standard}} \times V_{\text{Sample}}}{V_{\text{Sample}} \times C_{\text{Protein}}} \times \frac{OD_{\text{Sample}} - OD_{\text{Blank}}}{OD_{\text{Standard}} - OD_{\text{Blank}}} = \frac{5}{C_{\text{Protein}}} \times \frac{OD_{\text{Sample}} - OD_{\text{Blank}}}{OD_{\text{Standard}} - OD_{\text{Blank}}}$$

Citrate concentration per g of sample:

$$\text{Citrate } (\mu\text{mol/g}) = \frac{C_{\text{Standard}} \times V_{\text{Sample}}}{V_{\text{Sample}} \times V_{\text{Assay}} \times W} \times \frac{OD_{\text{Sample}} - OD_{\text{Blank}}}{OD_{\text{Standard}} - OD_{\text{Blank}}} = \frac{5}{W} \times \frac{OD_{\text{Sample}} - OD_{\text{Blank}}}{OD_{\text{Standard}} - OD_{\text{Blank}}}$$

Citrate concentration per ml serum or plasma:

$$\text{Citrate } (\mu\text{mol/ml}) = \frac{C_{\text{Standard}} \times V_{\text{Standard}}}{V_{\text{Standard}}} \times \frac{OD_{\text{Sample}} - OD_{\text{Blank}}}{OD_{\text{Standard}} - OD_{\text{Blank}}} = \frac{5}{W} \times \frac{OD_{\text{Sample}} - OD_{\text{Blank}}}{OD_{\text{Standard}} - OD_{\text{Blank}}}$$

Where:

$C_{\text{Protein}}$	Concentration of protein (in mg/ml)
$C_{\text{Standard}}$	Concentration of highest standard (5 mmol/L= 0.05 mmol/L)
$W$	Weight of the sample (in g)
$V_{\text{Assay}}$	Volume of Assay Buffer (1 ml)
$V_{\text{Sample}}$	Volume of sample (0.01 ml)
$V_{\text{Standard}}$	Volume of standard (0.01 ml)