Citrate Assay Kit

Catalog No.: abx298835

Size: 96 tests

Detection Range: 0.06 mmol/L – 2.0 mmol/L

Sensitivity: 0.06 mmol/L

Storage: Store at 4°C. Store the Reducing Reagent and Chromogenic Reagent in the dark.

Application: For quantitative detection of Citrate concentrations in serum, plasma, tissue homogenates, mitochondria samples, and other biological fluids.

Introduction: Citric acid, present as Citrate in biological systems, 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. Under acidic conditions, Cr^{6+} is reduced to Cr^{3+}. The reaction of citrate with Cr^{3+} produces a colored product which has an absorbance maximum at 545 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. Extraction Solution: 2 × 60 ml
3. Lysis Buffer: 20 ml
4. Reducing Reagent: 1 vial
5. Chromogenic Reagent: 2 × 1.5 ml
6. Standard (2 mmol/L): 2 ml
7. Plate Sealer: 2

Materials Required But Not Provided

1. Microplate reader (545 nm)
2. Centrifuge and microcentrifuge tubes
3. High-precision pipette and sterile pipette tips
4. Double distilled water
5. Timer
6. Ice
7. Sonicator
8. Mortar
9. Water bath

Protocol

A. Preparation of Samples and Reagents

1. Samples

Isolate the test samples soon after collecting and analyze immediately or aliquot and store at -20°C or -80°C for long-term storage. Avoid multiple freeze-thaw cycles.

The following sample preparation methods are intended as a guide and may be adjusted as required depending on the specific samples used.

- **Serum and Plasma:** Samples can be tested directly.
- **Tissue homogenates:** Add 0.9 ml of Extraction Solution to 0.1 g of sample and homogenize in an ice water bath. Centrifuge at 11,000 × g for 10 minutes at 4°C. Take the supernatant and keep on ice before carrying out the assay.
- **Mitochondria Samples:** Add 0.9 ml of Extraction Solution to 0.1 g of sample and homogenize in an ice water bath. Centrifuge at 600 × g for 5 minutes at 4°C. Transfer the supernatant to a fresh tube and centrifuge at 10,000 × g for 10 minutes at 4°C. Transfer the supernatant to a new tube – this solution can be discarded or used to separately determine the citrate content in the cytoplasm. To the pellet, add 200 µl of Lysis Buffer, dissolve fully with a vortex mixer, then centrifuge at 10,000 × g for 10 minutes at 4°C. Take the supernatant and stand on ice before assay. The protein concentration in the supernatant should be determined separately (**abx097193**).

It is recommended to carry out a preliminary experiment to determine the optimal dilution factor of samples before carrying out the formal experiment. The recommended dilution factor for different samples is as follows (for reference only):

Sample Type	Dilution Factor
Human Serum	3 – 15
Dog Serum	3 – 10
Rat Serum	3 – 15
Horse Serum	3 – 10
Mouse Plasma	3 – 10
10% Rat Brain Tissue Homogenate	5 – 10
10% Rat Liver Tissue Homogenate	5 – 20
10% Rat Kidney Tissue Homogenate	5 – 10
10% Rat Lung Tissue Homogenate	15 – 30
10% Mouse Heart Tissue Homogenate	5 – 20

Note:

- Fresh samples or recently obtained samples are recommended to prevent degradation and denaturalization that may lead to erroneous results.
- Lysis buffers may interfere with the kit. It is therefore recommended to use mechanical lysis methods for tissue homogenates.

2. Reagents

Bring all reagents to room temperature before use.

- **Reducing Reagent Working Solution**: Dissolve a vial of Reducing Reagent with 5 ml of Extraction Solution and mix thoroughly. Store at 4°C for up to 7 days in the dark.

- **Standards**: Label 8 tubes with 2.0, 1.5, 1.2, 1.0, 0.8, 0.5, 0.2 and 0 mmol/L. Dilute the 2 mmol/L Standard with double-distilled water to create concentrations of 0, 0.2, 0.5, 0.8, 1.0, 1.2, 1.5 and 2.0 mmol/L See the table for serial dilution reference.

Concentration (mmol/L)	**0**	**0.2**	**0.5**	**0.8**	**1.0**	**1.2**	**1.5**	**2.0**
2 mmol/L standard (µl)	0	20	50	80	100	120	150	200
Double-distilled water (µl)	200	180	150	120	100	80	50	0

For the blank, or 0 mmol/L standard, use pure double-distilled water. The volume of each standard will be 200 µl.

Note:

- If there is any precipitate in the Extraction Solution, heat the vial up to 80°C and swirl gently until the precipitate has fully dissolved. Allow the vial to cool fully back to room temperature before use.

B. Assay Procedure

Bring all kit components and samples to room temperature before use.

1. Set standard and sample tubes. *It is strongly recommended to prepare all tubes in duplicate.*
2. Add 30 µl of each standard dilution to the corresponding standard tubes.
3. Add 30 µl of sample into the sample tubes.
4. Add 210 µl of Extraction Solution to each tube.
5. Add 30 µl of Reducing Reagent Working Solution into each tube.
6. Add 30 µl of Chromogenic Reagent into each tube. Mix thoroughly and leave to stand at room temperature for 30 minutes.
7. Assign and record well plate positions for each standard and sample tube.
8. Add 200 µl of supernatant from each tube to the corresponding microplate wells. *Pipette samples up and down to mix before adding to wells. Avoid foaming or bubbles.*
9. Measure the OD values of each well at 545 nm using a microplate reader.

C. Calculations

The standard curve can be plotted as the absolute OD_{545} of each standard solution (y) vs. the respective concentration of the standard solution (x). A linear fit is recommended for the standard curve (y = ax + b). Create the standard curve with graph software. The Citrate concentration of the samples can be interpolated from the standard curve.

Serum and plasma samples:

$$\textbf{Citrate content (mmol/L)} = \frac{(\Delta \mathbf{A} - \mathbf{b})}{\mathbf{a}} \times \mathbf{f}$$

Tissue homogenate samples:

$$\textbf{Citrate content}\ (\mu\textbf{mol/g wet weight}) = \frac{(\Delta\mathbf{A} - \mathbf{b})}{\mathbf{a}} \times \frac{\mathbf{f} \times \mathbf{V}}{\mathbf{m}}$$

Mitochondria samples:

$$\textbf{Citrate content}\ (\textbf{mmol/g protein}) = \frac{(\Delta\mathbf{A} - \mathbf{b})}{\mathbf{a}} \times \frac{\mathbf{f}}{\mathbf{C_{Protein}}}$$

where:

$\Delta\mathbf{A}$	OD_{Sample} - $OD_{Blank.}$
$\mathbf{f}$	Dilution factor of sample before assay.
$\mathbf{m}$	Weight of tissue sample (0.1 g).
V	Volume of Extraction Solution (0.9 ml).
$\mathbf{C_{Protein}}$	Protein concentration of samples (g Protein/L)
$\mathbf{a}$	Gradient of the standard curve (y = **a**x + b)
b	Intercept of the standard curve (y = ax + **b**)

Technical Support

For troubleshooting and technical assistance, please contact us at support@abbexa.com.