Citrate Assay Kit

Catalog No.: abx295104

Size: 100 tests

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

Sensitivity: 0.05 mmol/L

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

Application: For detection and quantification of Citrate concentration in tissue homogenates, mitochondria samples, and other biological fluids.

Introduction

Abbexa's Citrate Assay Kit is a quick, convenient, and sensitive method for measuring and calculating Citrate concentration. In acidic conditions, Cr (VI) (Chromium) is reduced to Cr^{3+}. Cr^{3+} reacts with Citrate and forms a product with an absorbance maximum at 545 nm. The absorbance value is proportional to the Citrate concentration, which can then be calculated.

Kit components

1. Lysis Buffer: 20 ml
2. Chromogenic Reagent: 15 ml
3. Standard (1 mmol/L): 2 ml
4. Reducing Reagent: 1 vial
5. Buffer Solution: 4 × 45 ml

Materials required but not provided

1. Spectrophotometer (545 nm)
2. 1 ml quartz cuvettes
3. Double-distilled water
4. Pipette and pipette tips
5. 1.5 ml microcentrifuge tubes
6. Centrifuge
7. Vortex mixer
8. Water bath
9. Incubator

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.

- **Mitochondria (from tissue) Samples:** Carefully weigh 0.1 g of tissue, and add into 0.9 ml of Buffer Solution. Homogenize manually or mechanically in an ice water bath. Centrifuge at 600 × g for 5 minutes at 4°C. Take the supernatant and centrifuge at 11,000 × g for 10 minutes at 4°C. Discard the supernatant. Add 200 µl of Lysis Buffer and mix thoroughly using a vortex mixer to dissolve. Centrifuge at 11,000 × g for 10 minutes at 4°C. Collect the supernatant and keep on ice for detection.
- **Tissue Homogenates:** Carefully weigh 0.1 g of tissue, and add into 0.9 ml Buffer Solution. Homogenize manually or mechanically in an ice water bath. Collect the supernatant and centrifuge at 11,000 × g for 10 minutes at 4°C. Collect the supernatant and keep on ice for detection.
- **Other Biological Fluids:** Liquid samples can be tested directly.

  Note: To calculate Citrate concentration in Mitochondria Samples using the formulae in section **C. Calculation of Results**, the total protein concentration of the supernatant must 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. Dilute samples into different concentrations with Buffer Solution, then carry out the assay procedure. The recommended dilution factor for different samples is as follows (for reference only):

Sample Type	Dilution Factor
Human serum	4 - 6
10 % Mouse kidney tissue homogenate	2 – 4
10 % Rat kidney tissue homogenate	1
10 % Mouse heart tissue homogenate	2 - 4
10 % Mouse brain tissue homogenate	1
10 % Mouse liver tissue homogenate	1

Note:

- Fresh samples or recently obtained samples are recommended to prevent degradation and denaturalization that may lead to erroneous results.

2. Reagents

- **Reducing Reagent Working Solution:** Add 20 ml of Buffer Solution to one vial of Reducing Reagent and mix thoroughly to dissolve. Unused Reducing Reagent Working Solution can be stored at 4°C for up to 7 days.
- **Standard (0.25 mmol/L) Solution:** Prepare enough Standard (0.25 mmol/L) Solution for each standard tube required. Per tube, add 75 µl of double-distilled water to 25 µl of Standard (1 mmol/L) and mix thoroughly to prepare 100 µl of Standard (0.25 mmol/L) Solution. Prepare immediately before assaying.

Note:

- If there is any precipitate present in the Buffer Solution, heat the vial to 80°C and stir gently to dissolve the precipitate. Allow the tubes to cool back to room temperature before use.
- Incubate Buffer Solution in a water bath at 30°C for 30 minutes before use.

B. Assay Procedure

1. Mark microcentrifuge tubes for each standard, sample, and blank. *It is strongly recommended to prepare all the tubes in duplicate.*
2. Add 100 µl of sample to each sample tube.
3. Add 100 µl of Standard (0.25 mmol/L) Solution to the standard tube.
4. Add 100 µl of double-distilled water to the blank tube
5. Add 700 µl of Buffer Solution to each tube
6. Add 100 µl of Reducing Reagent Working Solution to each tube.
7. Add 100 µl of Chromogenic Reagent to each tube.
8. Mix thoroughly with a vortex mixer, then leave all tubes to stand at room temperature for 30 minutes.
9. Set the spectrophotometer to zero using double-distilled water.
10. Measure the OD of each tube at 545 nm with 1 ml quartz cuvettes.

C. Calculation of Results

1. Mitochondria Samples:

$$\text{Citrate content (µmol/mg Protein)} = \mathbf{F} \times \mathbf{c} \times \frac{(\mathrm{OD_{Sample}} - \mathrm{OD_{Blank}})}{(\mathrm{OD_{Standard}} - \mathrm{OD_{Blank}}) \times \mathrm{C_{Protein}}}$$

2. Tissue Samples:

$$\text{Citrate content (µmol/g fresh weight)} = \mathbf{F} \times \mathbf{c} \times \frac{(\mathrm{OD_{Sample}} - \mathrm{OD_{Blank}}) \times \mathbf{V}}{(\mathrm{OD_{Standard}} - \mathrm{OD_{Blank}}) \times \mathbf{W}}$$

3. Other Biological Fluids:

$$\text{Citrate content (mmol/L)} = \mathbf{F} \times \mathbf{c} \times \frac{(\mathrm{OD_{Sample}} - \mathrm{OD_{Blank}})}{(\mathrm{OD_{Standard}} - \mathrm{OD_{Blank}})}$$

where:

$\mathrm{OD_{Sample}}$	OD value of sample
$\mathrm{OD_{Standard}}$	OD value of standard
$\mathrm{OD_{Blank}}$	OD value of blank
V	Volume of Buffer Solution (0.9 ml)
$\mathrm{C_{Protein}}$	Concentration of protein in sample (mg Protein/ml)
W	The weight of the tissue sample (0.1 g)
F	The dilution factor of sample

Technical Support

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