Citrate Assay Kit

Catalog Number KA0864

100 assays

Version: 03

Intended for research use only

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Introduction

Background

Citric acid (HOOC-CH_2-C(-OH)(-COOH)-CH_2-COOH) is a key intermediate in the TCA cycle which occurs in mitochondria. It is formed by the addition of oxaloacetate to the acetyl group of acetyl-CoA derived from the glycolytic pathway. Citrate can be transported out of mitochondria and converted back to acetyl CoA for fatty acid synthesis. Citrate is an allosteric modulator of both fatty acid synthesis (acetyl-CoA carboxylase) and glycolysis (phospho- fructokinase). Citrate is widely used industrially in foods, beverages and pharmaceuticals. Citrate metabolism and disposition can vary widely due to sex, age and a variety of other factors. The Citrate Assay Kit provides a simple, sensitive and rapid means of quantifying citrate in a variety of samples. In the assay, citrate is converted to pyruvate via oxaloacetate. The pyruvate is quantified by converting a nearly colorless probe to an intensely colored (λmax=570 nm) and fluorescent (Ex/Em, 535/587 nm) product. The Citrate Assay Kit can detect 0.1 to 10 nmoles (~2 µM-10 mM) of citrate in a variety of samples.

General Information

Materials Supplied

List of component

Component	Amount
Citrate Assay Buffer	25 ml
Citrate Probe	0.2 ml
Citrate Enzyme Mix: lyophilized.	1 vial
Citrate Developer: lyophilized.	1 vial
Citrate Standard (10 µmol): lyophilized	1 vial

Storage Instruction

Store the kit at -20℃, protect from light. Warm the assay buffer to room temperature before use. Briefly centrifuge all small vials prior to opening.

Assay Protocol

Reagent Preparation

- Citrate Probe: Ready to use as supplied. Warm to 37℃ for 1-2 min to completely melt the DMSO solution before use. Store at -20℃, protect from light. Use within two months.
- Citrate Developer, Enzyme Mix: Dissolve with 220 µl Assay Buffer separately. Pipette up and down to dissolve. Aliquot into portions, store at -20℃. Avoid repeated freeze/thaw cycles. Use within 2 months.
- Citrate Standard: Dissolve in 100 µl dH_2O to generate 100 mM (100 nmol/µl) Citrate Standard solution. Keep on ice while in use. Store at -20℃

Assay Procedure

1. Standard Curve Preparations:
   Colorimetric Assay: Dilute the Citrate Standard to 1 nmol/µl by adding 10 µl of the Standard to 990 µl of dH_2O, mix well. Add 0, 2, 4, 6, 8, 10 µl into a series of standards wells on a 96 well plate. Adjust volume to 50 µl/well with Assay Buffer to generate 0, 2, 4, 6, 8, 10 nmol/well of the Standard.
   Fluorometric Assay: Dilute the Citrate standard to 0.1 nmol/µl by adding 10 µl of the standard to 990 µl of dH_2O, mix well, then further dilute by adding 10 µl to 90 µl of dH_2O. Add 0, 2, 4, 6, 8, 10 µl into a series of standards well on a 96-well plate. Adjust the volume to 50 µl/well to generate 0, 0.2, 0.4, 0.6, 0.8, 1.0 nmol/well.
2. Sample Preparation:
   Tissue (20 mg) or cells ($2x10^6$) should be rapidly homogenized with 100 µl of Citrate Assay Buffer. Centrifuge at 15,000 g for 10 minutes to remove cell debris. Enzymes in samples may interfere with the assay. We suggest deproteinizing samples using a perchloric acid/KOH protocol or 10 kd molecular weight cut off spin columns. Add 1-50 µl samples into duplicate wells of a 96-well plate and bring volume to 50 µl with Assay Buffer. We suggest testing several doses of your samples to ensure readings are within the standard curve range.
3. Develop:
   Mix enough reagent for the number of samples and standards to be performed: For each well, prepare a total 50 µl Reaction Mix containing:

	Colorimetric Assay		Fluorometric Assay	
	Sample	Bkgd Control*	Sample	Bkgd Control*
Citrate Assay Buffer	44 µl	46 µl	44 µl	46 µl
Citrate Enzyme Mix	2 µl	-	2 µl	-
Developer	2 µl	2 µl	2 µl	2 µl
Citrate Probe**	2 µl	2 µl	2 µl	2 µl

*Samples may contain oxaloacetate or pyruvate which can generate a background and need to be subtracted from the sample background signal.

**For the fluorometric assay, dilute 10X with DMSO to reduce fluorescent background.

4. Add 50 µl of the Reaction Mix to each well containing the Citrate Standard and test samples. Add 50 µl of the sample background control mix to background control wells.
5. Incubate for 30 minutes at room temperature, protect from light.
6. Measure OD at 570 nm or fluorescence at Ex/Em 535/587nm.

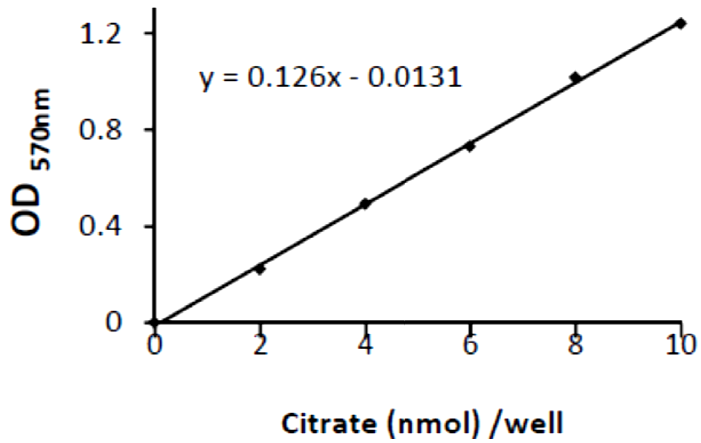
Data Analysis

Calculation of Results

Correct background by subtracting the value of the 0 Citrate Standard from all readings. Next subtract the value of the Sample Background Control from the test sample. Plot the standard curve. Apply corrected sample readings to the standard curve to get Citrate amount in the sample wells. The Citrate concentrations in the test samples equals:

C = Ay/Sv (nmol/µl; or µmol/ml; or mM)

Where:
Ay is the amount of citrate (nmol) in your sample from the standard curve.
Sv is the sample volume (µl) added to the sample well.
Citric acid molecular weight: 191 g/mol.



Citrate standard curve generated using this kit protocol