MA-0139

Citrate Assay

(100 wells, Colorimetric, OD 570 nm or Fluorometric, Ex/Em = 535/587 nm, Store at -20°C)

Introduction:

Citrate is a metabolic intermediate in the tricarboxylic acid cycle, a key metabolic pathway, responsible for generating energy in the form of ATP, which occurs in mitochondria. It is formed by the condensation of oxaloacetate and acetyl CoA, derived from the glycolytic pathway. Citrate can either be immediately converted to isocitrate and continue along the TCA cycle or transported from mitochondria to the cytoplasm where it is converted back to acetyl CoA for fatty acid synthesis. Citrate is an allosteric modulator of fatty acid synthesis and glycolysis. Decreased citrate levels are associated with kidney stones, metabolic syndrome and prostate cancer. AkrivisBio's Citrate Assay provides a simple, sensitive means of measuring citrate in a variety of samples with a sensitivity below 0.1 nanomoles.

Assay Principle:

1 – Citrate is converted to oxaloacetate by the action of citrate lyase in the presence of ATP and Coenzyme A
2 – Oxaloacetate is converted to pyruvate by oxaloacetate decarboxylase
3 – Pyruvate is oxidized by pyruvate oxidase forming hydrogen peroxide.
4 – Peroxidase utilizes the peroxide to convert ADHP to resorufin with the formation of intense color and fluorescence.

Assay Components:

Assay Buffer	25 ml	WM	MA-0139-A
ADHP Solution	0.2 ml	Red	MA-0139-B
Citrate Lyase	lyoph	Purple	MA-0139-C
Enzyme Mix	lyoph	Green	MA-0139-D
(Oxaloacetate Decarboxylase, Pyruvate Oxidase, Peroxidase)			
Citrate Standard	lyoph	Yellow	MA-0139-E

Storage and Handling:

Store unopened assay at -20°C. Thaw buffer and ADHP at room temperature before use. Centrifuge all small vials briefly, prior to opening.
Assay Buffer: Ready to use as supplied. Store at 4°C.
ADHP Solution: Ready to use as supplied. Store at -20°C.
Citrate Lyase, Enzyme Mix: Dissolve each with 220 µl Assay Buffer. If assay is to be used repeatedly over a period of time, aliquot into convenient portions to avoid repeated freeze/thaw cycles and store at -20°C.
Citrate Standard: Dissolve in 100 µl dH_2O giving 40 mM Citrate solution. Keep on ice while in use. Store at -20°C.

Assay Protocol:

1. Standard Curve:

Absorbance-based Assay: Dilute the Citrate Standard to 0.4 mM by transferring 10 µl of the Standard to 990 µl of DI H_2O. Transfer 0 – 5 – 10 – 15 – 20 – 25 µl to a series of wells in a 96 well plate. Adjust all well volumes to 50 µl with Assay Buffer, giving 0 – 2 – 4 – 6 – 8 – 10 nmol/well.

Fluorescence-based Assay: Dilute the Citrate standard as for the absorbance-based assay, then further dilute to 40 µM by transferring 10 µl to 90 µl of DI H_2O. Transfer 0 – 5 – 10 – 15 – 20 - 25 µl into a series of wells in a 96-well plate. Adjust all well volumes to 50 µl giving 0 – 0.2 – 0.4 – 0.6 – 0.8 – 1.0 nmol/well.

2. Sample Preparation: Homogenize tissue (10 mg) or cells (10^6) with 100 µl of Assay Buffer. Centrifuge at 16,000 X g for 5 min to remove cell debris. Enzymes in samples may interfere with the assay. Deproteinize with a protein precipitant or 10 kDa MWCO spin filter Add 2-50 µl sample to wells in duplicate in a 96-well plate. Adjust all well volumes to 50 µl with Assay Buffer. Use one of the paired samples as background control.

3. Initiate Reaction: Each well will require 50 µl of Reaction Mix (samples) or Background Control Mix (Background Controls). Prepare enough of each reagent for the number of wells to be tested containing:

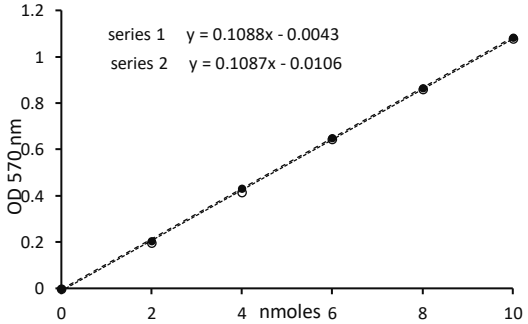
	Colorimetric Assay		Fluorometric Assay	
	Sample	**Background Control**	**Sample**	**Background Control**
Assay Buffer	44 µl	46 µl	45.6 µl	47.6 µl
Citrate Lyase	2 µl	-----	2 µl	-----
Enzyme Mix	2 µl	2 µl	2 µl	2 µl
ADHP Solution	2 µl	2 µl	0.4 µl	0.4 µl

Add 50 µl of the Reaction Mix to each well containing Standard or test sample. Add 50 µl of the background control mix to background control wells.

4. Measurement: Monitor wells at room temperature, using absorbance at 570 nm or fluorescence at 535 nm excitation/ 587 nm emission.

5. Typical Results:

nmol Standard	Standard Raw Values (OD)		Background Corrected Values (OD)	
0	0.0466	0.0479	0	0
2	0.2527	0.2455	0.2061	0.1976
4	0.479	0.4634	0.4324	0.4155
6	0.6968	0.6937	0.6502	0.6458
8	0.9129	0.9090	0.8663	0.8611
10	1.1304	1.1260	1.0838	1.0781



6. Calculation: Subtract the value of the 0 Citrate Standard from all standards. Plot the Standard Curve and determine the slope of the standard curve. This value defines the sensitivity of the assay to citrate and will be used to determine citrate in the wells. Subtract the value of the Background Control wells from the paired sample well. Divide the background corrected values by the slope of the standard curve to give nmoles of citrate in the wells. To convert this value back to amount of citrate in the original samples:

A. Divide the nmoles citrate in the well by the volume in µl of sample added to the well = nmoles citrate / µl sample
B. Multiply by the total volume of deproteinized sample = total nmoles citrate in sample.
C. Divide total citrate in sample by mg of tissue (or # of cells, etc.) = nmoles of citrate per mg tissue (per # of cells, etc.)

FOR RESEARCH USE ONLY. Not to be used for diagnostic purposes.