Product Information

Citrate Colorimetric/Fluorometric Assay Kit

I. Kit Contents:

Components	K2207-100 100 assays	Cap Color	Part Number
Citrate Assay Buffer	25 ml	WM	K2207-C-1
Citrate Probe	0.2 ml	Red	K2207-C-2
Citrate Enzyme Mix	lyophilized	Purple	K2207-C-3
Citrate Developer	lyophilized	Green	K2207-C-4
Citrate Standard (10 µmol)	lyophilized	Yellow	K2207-C-5

II. Introduction:

Citrate is an important intermediate in the citric acid cycle which occurs in mitochondria. Citrate is produced by the addition of oxaloacetate to the acetyl group of acetyl-CoA derived from the glycolytic pathway. Citrate can also be transported out of mitochondria and transformed to acetyl CoA for fatty acid synthesis. Citrate is an allosteric modulator of both phospho-fructokinase (glycolysis) and acetyl-CoA carboxylase (fatty acid synthesis). Citrate metabolism and disposition is various due to age, sex and a variety of other factors.

The Citrate Colorimetric/Fluorometric Assay Kit provides a sensitive, simple, fast and convenient way for detection of citrate levels in various biological samples based on colorimetric and fluorometric method. In the assay, citrate is converted to pyruvate via oxaloacetate. The formed pyruvate is quantified via converting a nearly colorless probe to an intensely fluorescent (Ex/Em, 535/587 nm) and colored (570 nm) product. The kit can detect 0.1 to 10 nmoles (~ 2 µM-10 mM) citrate in various samples.

III. Storage and Handling:

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

IV. Reagent Preparation and Storage Conditions:

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℃.

V. Assay Protocol:

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 wells 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 (2 x 10^6) should be rapidly homogenized with 100 µl of Citrate Assay Buffer. Centrifuge at 15,000 g for 10 min to remove cell debris. Enzymes in samples may interfere with the assay. We suggest deproteinizing samples using a perchloric acid/KOH protocol or 10 kDa molecular weight cut off spin columns. Add 1 - 50 µl sample 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 min at room temperature, protect from light.

6. Measure OD at 570 nm or fluorescence at Ex/Em 535/587nm.

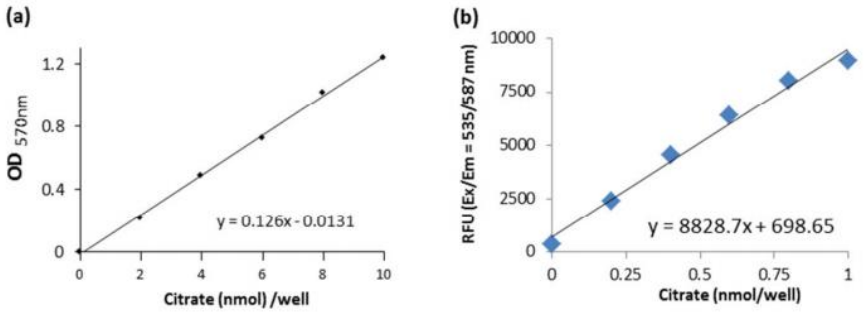
7. Calculation: Correct background by subtracting the value of the 0 Citrate Standard from all readings. Next subtract the value of the Sample Background Control from the test sample. Plot the standard curve. Apply corrected sample readings to the standard curve to get Citrate amount in the sample wells.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

General Troubleshooting Guide:

Problems	Cause	Solution
Assay not working	• Use of a different buffer • Omission of a step in the protocol • Plate read at incorrect wavelength	• Assay buffer must be at room temperature • Refer and follow the data sheet precisely • Check the wavelength in the data sheet and the filter settings

	• Use of a different 96-well plate	of the instrument • Fluorescence: Black plates ; Luminescence: White plates; Colorimeters: Clear plates
Samples with erratic readings	• Use of an incompatible sample type • Samples prepared in a different buffer • Samples were not deproteinized (if indicated in d atasheet) • Cell/ tissue samples were not completely homogenized • Samples used after multiple free-thaw cycles • Presence of interfering substance in the sample • Use of old or inappropriately stored samples	• Refer data sheet for details about incompatible samples • Use the assay buffer provided in the kit or refer data sheet for instructions • Use the 10 kDa spin cut-off filter or PCA precipitation as indicated • Use Dounce homogenizer (increase the number of strokes); observe for lysis under microscope • Aliquot and freeze samples if needed to use multiple times • Troubleshoot if needed, deproteinize samples • Use fresh samples or store at correct temperatures till use
Lower/ Higher readings in Samples and Standards	• Improperly thawed components • Use of expired kit or improperly stored reagents • Allowing the reagents to sit for extended times on ice • Incorrect incubation times or temperatures • Incorrect volumes used	• Thaw all components completely and mix gently before use • Always check the expiry date and store the components appropriately • Always thaw and prepare fresh reaction mix before use • Refer data sheet & verify correct incubation times and temperatures • Use calibrated pipettes and aliquot correctly
Readings do not follow a linear pattern for Standard curve	• Use of partially thawed components • Pipetting errors in the standard • Pipetting errors in the reaction mix • Air bubbles formed in well • Standard stock is at an incorrect concentration • Calculation errors • Substituting reagents from older kits/ lots	• Thaw and resuspend all components before preparing the reaction mix • Avoid pipetting small volumes • Prepare a master reaction mix whenever possible • Pipette gently against the wall of the tubes • Always refer the dilutions in the data sheet • Recheck calculations after referring the data sheet • Use fresh components from the same kit
Unanticipated results	• Measured at incorrect wavelength • Samples contain interfering substances • Use of incompatible sample type • Sample readings above/below the linear range	• Check the equipment and the filter setting • Troubleshoot if it interferes with the kit • Refer data sheet to check if sample is compatible with the kit or optimization is needed • Concentrate/ Dilute sample so as to be in the linear range
Note: The most probable list of causes is under each problem section. Causes/ Solutions may overlap with other problems.		

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Our promise

If the product does not perform as described on this datasheet, we will offer a refund or replacement. For more details, please visit http://www.apexbt.com/ or contact our technical team.

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