

Product Information

Citrate Assay Kit

Catalog Number **MAK057**

Storage Temperature -20°C

TECHNICAL BULLETIN

Product Description

Citrate 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. Citrate is an allosteric modulator of both fatty acid synthesis via its actions on acetyl-CoA carboxylase and of glycolysis via its actions on phosphofructokinase. Citrate metabolism and disposition can vary widely due to sex, age, and a variety of other factors including disease states. Cellular citrate levels are decreased in prostate cancer cells and citrate levels may be a marker of prostate cancer growth rate.

The Citrate Assay Kit provides a simple, sensitive, and rapid means of quantifying citrate in a variety of samples. Citrate concentration is determined by a coupled enzyme assay, which results in a colorimetric (570 nm)/fluorometric ($\lambda_{\text{ex}} = 535/\lambda_{\text{em}} = 587$ nm) product, proportional to the citrate present. Typical detection range of the Citrate Assay Kit is 0.2–10 nmoles of citrate in a variety of samples.

Components

The kit is sufficient for 100 assays in 96 well plates.

Citrate Assay Buffer Catalog Number MAK057A	25 mL
Citrate Probe in DMSO Catalog Number MAK057B	0.2 mL
Citrate Enzyme Mix Catalog Number MAK057D	1 vL
Citrate Developer Catalog Number MAK057E	1 vL
Citrate Standard, 10 μmole Catalog Number MAK057F	1 vL

Reagents and Equipment Required but Not Provided.

- 96 well flat-bottom plate – It is recommended to use black plates with clear bottoms for fluorescence assays and clear plates for colorimetric assays.
- Fluorescence or spectrophotometric multiwell plate reader.
- 10 kDa Molecular Weight Cut-Off (MWCO) Spin Filter

Precautions and Disclaimer

This product is for R&D use only, not for drug, household, or other uses. Please consult the Material Safety Data Sheet for information regarding hazards and safe handling practices.

Preparation Instructions

Briefly centrifuge vials before opening. Use ultrapure water for the preparation of reagents. To maintain reagent integrity, avoid repeated freeze/thaw cycles.

Citrate Assay Buffer – Allow buffer to come to room temperature before use.

Citrate Probe Solution – Warm to room temperature to thaw the solution prior to use. Aliquot and store any remaining solution at -20°C protected from light and moisture. Upon thawing, the Citrate Probe is ready-to-use in the colorimetric assay.

For the fluorescence assay, dilute an aliquot of the Citrate Probe Solution 5 to 10-fold with Citrate Assay Buffer, just prior to use. This will reduce the background of the fluorescence assay.

Citrate Enzyme Mix and Citrate Developer – Reconstitute in 220 μL of Citrate Assay Buffer. Mix well by pipetting, then aliquot and store at -20°C . Use within 2 months of reconstitution.

Citrate Standard - Reconstitute in 100 μL of water to generate a 100 mM (100 nmole/ μL) Citrate Standard Solution. Mix well by pipetting, then aliquot and store at -20°C .

Storage/Stability

The kit is shipped on wet ice and storage at -20°C , protected from light, is recommended.

Procedure

All samples and standards should be run in duplicate.

Citrate Standards for Colorimetric Detection

Dilute 10 μL of the 100 mM Citrate Standard Solution with 990 μL of water to generate a 1 mM standard solution. Add 0, 2, 4, 6, 8, and 10 μL of the 1 mM citrate standard into a 96 well plate, generating 0 (blank), 2, 4, 6, 8, and 10 nmole/well standards. Add Citrate Assay Buffer to each well to bring the volume to 50 μL .

Citrate Standards for Fluorometric Detection

Prepare a 1 mM standard solution as for the colorimetric assay. Take 10 μL of the 1 mM standard and add to 90 μL of water to make a 0.1 mM standard solution. Add 0, 2, 4, 6, 8, and 10 μL of the 0.1 mM citrate standard into a 96 well plate, generating 0 (blank), 0.2, 0.4, 0.6, 0.8, and 1.0 nmole/well standards. Add Citrate Assay Buffer to each well to bring the volume to 50 μL .

Sample Preparation

Both the colorimetric and fluorometric assays require 50 μL of sample for each reaction (well).

Tissue (20 mg) or cells (1×10^6) should be rapidly homogenized with 100 μL of the Citrate Assay Buffer. Centrifuge the samples at $15,000 \times g$ for 10 minutes to remove insoluble material. Bring samples to a final volume of 50 μL with Citrate Assay Buffer.

Note: Because enzymes in samples may interfere with the assay, samples should be deproteinized with a 10 kDa MWCO spin filter prior to addition to the reaction.

Diluted samples can be directly added to the well. For unknown samples, it is suggested to test several sample volumes to make sure the readings are within the standard curve range.

Assay Reaction

1. Set up the Reaction Mixes according to the scheme in Table 1.

Note: Oxaloacetate or pyruvate in samples can cause background in the assay. To remove the oxaloacetate or pyruvate background, include a blank sample for each sample by omitting the Citrate Enzyme Mix. The background control readings can then be subtracted from the sample readings.

Table 1.
Reaction Mixes

Reagent	Samples and Standards	Blank Sample
Citrate Assay Buffer	44 μL	46 μL
Citrate Enzyme Mix	2 μL	–
Citrate Developer	2 μL	2 μL
Citrate Probe	2 μL	2 μL

2. Add 50 μL of the appropriate Reaction Mix to each of the standard, sample, and blank control wells. Mix well using a horizontal shaker or by pipetting, and incubate at room temperature for 30 minutes, protected from light.
3. For colorimetric assays, measure the absorbance at 570 nm (A_{570}). For fluorometric assays, measure fluorescence intensity ($\lambda_{\text{ex}} = 535/\lambda_{\text{em}} = 587 \text{ nm}$).

Results

Calculations

The background for either assay is the value obtained for the 0 (blank) Citrate standard. Correct for the background by subtracting the blank value from all readings. Background values can be significant and must be subtracted from all readings.

Use the values obtained from the citrate standards to plot a standard curve. The amount of citrate present in the samples may be determined from the standard curve.

Note: A new standard curve must be set up each time the assay is run.

Concentration of Citrate

$$S_a/S_v = C$$

S_a = Amount of citrate in unknown sample (nmole) from standard curve

S_v = Sample volume (μL) added into the wells.

C = Concentration of citrate in sample

Citric Acid molecular weight: 191.12 g/mole

Sample Calculation

Citrate amount (S_a) = 5.84 nmole

Assay volume (S_v) = 50 μL

$$5.84 \text{ nmole}/50 \mu\text{L} = 0.1169 \text{ nmole}/\mu\text{L}$$

$$0.1169 \text{ nmole}/\mu\text{L} \times 191.12 \text{ ng/nmole} = 22.34 \text{ ng}/\mu\text{L}$$

Troubleshooting Guide

Problem	Possible Cause	Suggested Solution
Assay not working	Cold assay buffer	Assay Buffer must be at room temperature
	Omission of step in procedure	Refer and follow Technical Bulletin precisely
	Plate reader at incorrect wavelength	Check filter settings of instrument
	Type of 96 well plate used	For fluorescence assays, use black plates with clear bottoms. For colorimetric assays, use clear plates
Samples with erratic readings	Samples prepared in different buffer	Use the Assay Buffer provided
	Samples were not deproteinized	Use a 10 kDa MWCO spin filter to deproteinize samples
	Cell/Tissue culture samples were incompletely homogenized	Repeat the sample homogenization, increasing the length and extent of homogenization step.
	Samples used after multiple freeze-thaw cycles	Aliquot and freeze samples if samples will be used multiple times
	Presence of interfering substance in the sample	If possible, dilute sample further
	Use of old or inappropriately stored samples	Use fresh samples and store correctly until use
Lower/higher readings in samples and standards	Improperly thawed components	Thaw all components completely and mix gently before use
	Use of expired kit or improperly stored reagents	Check the expiration date and store the components appropriately
	Allowing the reagents to sit for extended times on ice	Prepare fresh Reaction Mix before each use
	Incorrect incubation times or temperatures	Refer to Technical Bulletin and verify correct incubation times and temperatures
	Incorrect volumes used	Use calibrated pipettes and aliquot correctly
Non-linear standard curve	Use of partially thawed components	Thaw and resuspend all components before preparing the reaction mix
	Pipetting errors in preparation of standards	Avoid pipetting small volumes
	Pipetting errors in the Reaction Mix	Prepare a Reaction Mix whenever possible
	Air bubbles formed in well	Pipette gently against the wall of the plate well
	Standard stock is at incorrect concentration	Refer to the standard dilution instructions in the Technical Bulletin
	Calculation errors	Recheck calculations after referring to Technical Bulletin
	Substituting reagents from older kits/lots	Use fresh components from the same kit
Unanticipated results	Samples measured at incorrect wavelength	Check the equipment and filter settings
	Samples contain interfering substances	If possible, dilute sample further
	Sample readings above/below the linear range	Concentrate or dilute samples so readings are in the linear range

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