Citrate Assay Kit (Colorimetric/Fluorometric)

LS-K178-100 (100 Tests) • **Store at -20°C**

LSBio
LifeSpan BioSciences, Inc.

Introduction

CITRATE is an intermediate in the citric acid cycle and is involved in fatty acid synthesis. LSBio's Citrate Assay Kit provides a simple, and automation-ready procedure for measuring citrate concentration. Citrate is converted into pyruvate which is then oxidized with the conversion of the dye into a colored and fluorescent form. The color intensity at 570 nm or fluorescence intensity at λex/em = 530/585 nm is directly proportional to the citrate concentration in the sample.

Key Features

- Fast and sensitive. Linear detection range: 4 to 400 µM citrate for colorimetric assays and 0.5 to 40 µM for fluorometric assays.
- Convenient and high-throughput. Homogeneous "mix-incubate-measure" type assay. Can be readily automated on HTS liquid handling systems for processing thousands of samples per day.

Applications

- Citrate determination in biological samples (e.g. plasma, serum, urine, tissue and culture media.)

Components

Component	K178-100
	100 Tests
Developer	10 mL
CL Enzyme	Dried
ODC Enzyme	120 µL
Citrate Standard	500 µL
Dye Reagent	120 µL

Materials Not Supplied

Pipetting devices, clear or black flat-bottom 96-well plates, plate reader or centrifuge tubes.

Storage

The kit is shipped on ice. Store all kit components at -20 °C.

Assay Procedure

Reagent Preparation

Dissolve the CL Enzyme with 120 µL Developer. Pipette up and down to assure the enzyme is fully dissolved. Reconstituted CL enzyme is stable for 4 weeks stored at -20°C. Before each use of the CL Enzyme, pipette up and down to assure the enzyme is resuspended.

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Sample Preparation

Tissue or cell samples (2 x10^6) can be homogenized in 100 µL PBS. Centrifuge at 14,000 rpm for 5 min. Use clear supernatant for assay. If planning to measure citrate in culture media, avoid media with high pyruvate concentrations (e.g. DMEM, L-15, F12, etc.).

Serum and plasma samples should be deproteinated using a 10 kDa spin filter (e.g. Amicon Ultra-0.5). Alternatively, untreated serum and plasma can measured directly if an internal standard is used.

Urine samples should be diluted at least 5-fold and an internal standard should be used.

Colorimetric Procedure

1. Standards. Dilute the Citrate Standard to 400 µM by mixing 10 µL 10 mM Standard with 240 µL dH2O. Next, dilute standards in 1.5-mL centrifuge tubes as described in the table. If assaying culture media with phenol red, dilute the Citrate Standard in culture media.

No	Premix + dH_2O	Citrate (µM)
1	100 µL + 0 µL	400
2	60 µL + 40 µL	240
3	30 µL + 70 µL	120
4	0 µL + 100 µL	0

Transfer 20 µL of each standard to separate wells in a 96 well plate.

2. Samples. Add 20 µL of each sample to two separate wells in a 96 well plate (each sample requires a sample blank).

If using an internal standard, samples will need three separate reactions: 1) sample plus standard, 2) sample alone and 3) sample blank. For the internal standard prepare 500 µL 1000 µM citrate standard by mixing 50 µL 10 mM Standard and 450 µL dH2O. For the sample plus standard well, add 5 µL 1000 µM citrate and 20 µL sample. For the sample and sample blank wells, add 5 µL dH2O and 20 µL sample.

3. Citrate Detection. Prepare enough working reagent (WR) for all standards and samples. For each reaction combine the following: 85 µL Developer, 1 µL CL Enzyme, 1 µL ODC Enzyme, and 1 µL Dye Reagent. For the Sample Blanks, prepare a WR without the CL Enzyme. Add 80 µL of the appropriate WR to each Standard and Sample well. Mix well and incubate protected from light for 15 min at RT.

4. Read OD570nm.

Fluorometric Procedure

For fluorometric assays, the linear detection range is 1 to 40 µM citrate. Dilute the standards prepared in Colorimetric Procedure 1:10 in dH_2O. If an internal standard is used, use 5 µL of 100 µM citrate.

Transfer 20 µL standards and 20 µL samples (2 wells per sample if a standard curve is used; 3 wells per sample if an internal standard is used, see Colorimetric Procedure) into separate wells of a black 96-well plate.

Add 80 µL of appropriate Working Reagent (see Colorimetric Procedure) to each well. Tap plate to mix.

Incubate protected from light for 15 min at at RT and read fluorescence at $\lambda_{ex/em}$ = 530/585 nm.

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Calculations

Subtract the blank value (#4) from the standard values and plot the ΔOD or ΔF against standard concentrations. Determine the slope and calculate the citrate concentration of the Samples as follows:

$$[\text{Citrate}] = \frac{R_{SAMPLE} - R_{BLANK}}{\text{Slope } (\mu M^{-1})} \times n \quad (\mu M)$$

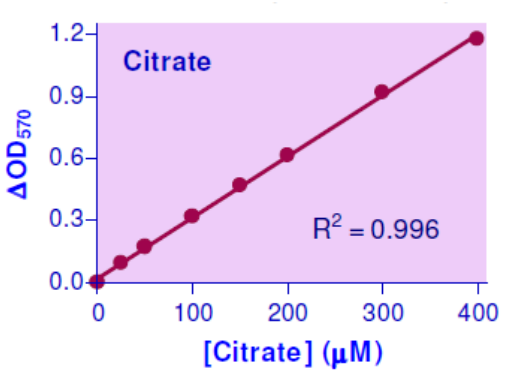
If an internal standard was used, the sample citrate concentration is computed as follows:

$$[\text{Citrate}] = \frac{R_{SAMPLE} - R_{BLANK}}{R_{STANDARD} - R_{SAMPLE}} \times \frac{[\text{Standard}]}{4} \times n \quad (\mu M)$$

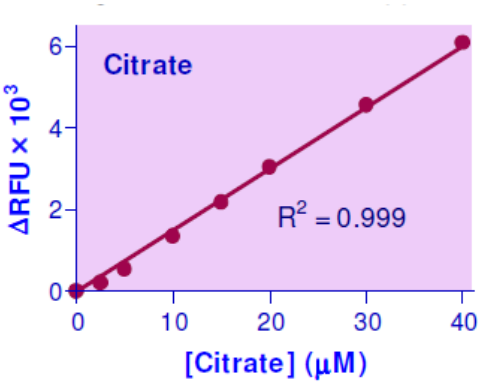
where R_{SAMPLE}, R_{BLANK} and $R_{STANDARD}$ are OD or fluorescence readings of the Sample, Sample Blank and the Sample plus Standard respectively. *n* is the sample dilution factor. Notes: The volume of the internal standard is 4× lower than the sample volume; thus, the internal standard concentration should be divided by 4. If the calculated citrate concentration is >400 µM for the colorimetric assay, or >40 µM for the fluorometric assay, dilute sample in dH_2O and repeat assay. Multiply result by the dilution factor *n*.

Conversions: 100 µM citrate equals 19.1 mg/L, 0.0019% or 19.1 ppm.

Sample Data



96-well colorimetric assay



96-well fluorimetric assay

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