EnzyChrom™ Citrate Assay Kit (ECIT-100)

Quantitative Colorimetric/Fluorimetric Citrate Determination

DESCRIPTION

CITRATE is an intermediate in the citric acid cycle and is involved in fatty acid synthesis. BioAssay Systems' 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 $\lambda_{ex/em}$ = 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 fluorimetric 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.)

KIT CONTENTS (100 TESTS IN 96-WELL PLATES)

Developer: 10 mL **CL Enzyme:** Dried
Dye Reagent: 120 µL **ODC Enzyme**: 120 µL
Citrate Standard: 500 µL

Storage conditions. The kit is shipped on ice. Store all kit components at -20 °C. Shelf life of six months after receipt.
Precautions: reagents are for research use only. Normal precautions for laboratory reagents should be exercised while using the reagents. Please refer to Material Safety Data Sheet for detailed information.

PROCEDURES

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.

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 dH_2O. 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 dH_2O. 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 dH_2O 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 OD_{570nm}.

Fluorimetric Procedure

For fluorimetric 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.

CALCULATION

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:

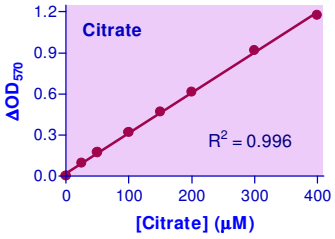
$$[\text{Citrate}] = \frac{R_{SAMPLE} - R_{BLANK}}{\text{Slope } (\mu M^{-1})} \times n \quad (\mu M)$$

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

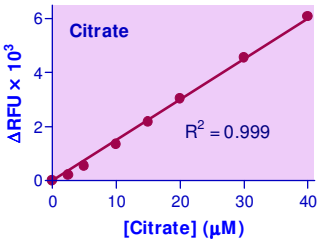
$$[\text{Citrate}] = \frac{R_{SAMPLE} - R_{BLANK}}{R_{STANDARD} - R_{SAMPLE}} \times \frac{[\text{Standard}]}{4} \times n \quad (\mu M)$$

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 fluorimetric 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.



96-well colorimetric assay



96-well fluorimetric assay

MATERIALS REQUIRED, BUT NOT PROVIDED

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

PUBLICATIONS

1. Pant, A. et al (2021). Viral growth factor-and STAT3 signaling-dependent elevation of the TCA cycle intermediate levels during vaccinia virus infection. PLoS Pathogens, 17(2).
2. Fu, X., et al (2018). Runx2/Osterix and zinc uptake synergize to orchestrate osteogenic differentiation and citrate containing bone apatite formation. Advanced Science 5.4: 1700755.
3. Trivedi, A. K., et al. (2015). Adaptation of oxidative phosphorylation to photoperiod-induced seasonal metabolic states in migratory songbirds. Comparative Biochemistry and Physiology Part A: Molecular & Integrative Physiology 184): 34-40.