

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 μ M citrate for colorimetric assays and 0.5 to 40 μ M 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 μ L **ODC Enzyme:** 120 μ L
Citrate Standard: 500 μ L

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 μ L 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 x 10⁶) can be homogenized in 100 μ L 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 be 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 μ M by mixing 10 μ L 10 mM Standard with 240 μ L 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 ₂ O	Citrate (μ M)
1	100 μ L + 0 μ L	400
2	60 μ L + 40 μ L	240
3	30 μ L + 70 μ L	120
4	0 μ L + 100 μ L	0

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

2. **Samples.** Add 20 μ L 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 μ L 1000 μ M citrate standard by mixing 50 μ L 10 mM Standard and 450 μ L dH₂O. For the sample plus standard well, add 5 μ L 1000 μ M citrate and 20 μ L sample. For the sample and sample blank wells, add 5 μ L dH₂O and 20 μ L sample.

3. **Citrate Detection.** Prepare enough working reagent (WR) for all standards and samples. For each reaction combine the following: 85 μ L Developer, 1 μ L CL Enzyme, 1 μ L ODC Enzyme, and 1 μ L Dye Reagent. For the Sample Blanks, prepare a WR **without** the CL Enzyme. Add 80 μ L 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 μ M citrate. Dilute the standards prepared in *Colorimetric Procedure* 1:10 in dH₂O. If an internal standard is used, use 5 μ L of 100 μ M citrate.

Transfer 20 μ L standards and 20 μ L 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 μ L of appropriate Working Reagent (see *Colorimetric Procedure*) to each well. Tap plate to mix.

Incubate protected from light for 15 min 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 Δ OD or Δ F against standard concentrations. Determine the slope and calculate the citrate concentration of the Samples as follows:

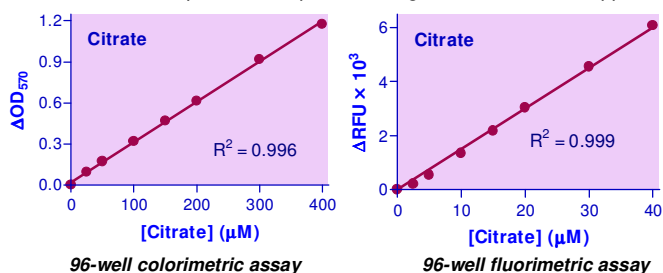
$$[\text{Citrate}] = \frac{R_{\text{SAMPLE}} - R_{\text{BLANK}}}{\text{Slope } (\mu\text{M}^{-1})} \times n \quad (\mu\text{M})$$

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

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

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 4x lower than the sample volume; thus, the internal standard concentration should be divided by 4. If the calculated citrate concentration is >400 μ M for the colorimetric assay, or >40 μ M for the fluorimetric assay, dilute sample in dH₂O and repeat assay. Multiply result by the dilution factor *n*.

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



MATERIALS REQUIRED, BUT NOT PROVIDED

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

LITERATURE

- Tompkins, D and Toffaletti, J (1982). Enzymatic Determination of Citrate in Serum and Urine, with Use of the Worthington "Ultrafree" Device. Clin. Chem. 28:192-5.
- Costello, LC and O'Neill, JJ (1969). A simplified and sensitive method for citrate determination in biological samples. J Appl Physiol. 27: 120-2.
- Parvin, R et al (1980). Convenient rapid determination of picomole amounts of oxaloacetate and citrate. Anal. Biochem. 104: 296-9.