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Product Information

Citrate Assay Kit

Catalog Number **MAK333**
Storage Temperature –20 °C

TECHNICAL BULLETIN

Product Description

Citrate is an intermediate in the citric acid cycle and is involved in fatty acid synthesis. The 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, fluorescent form. The color intensity at 570 nm or fluorescence intensity at λ_{ex} = 530 nm/λ_{em} = 585 nm is directly proportional to the citrate concentration in the sample.

The Citrate Assay Kit has a linear detection range of 4–400 μM citrate for the colorimetric assay and 0.5–40 μM for the fluorimetric assay.

Suitable for citrate determination in biological samples (e.g. plasma, serum, urine, tissue and culture media.)

Components

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

Developer Catalog Number MAK333A	10 mL
CL Enzyme (Dried) Catalog Number MAK333B	1 Vial
Dye Reagent Catalog Number MAK333C	120 μL
ODC Enzyme Catalog Number MAK333D	120 μL
Citrate Standard (10 mM) Catalog Number MAK333E	500 μL

Reagents and Equipment Required but Not Provided.

- Pipetting devices and accessories (e.g., multichannel pipettor)
- Centrifuge tubes
- 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
- Reagents and equipment for sample preparation

Precautions and Disclaimer

This product is for Research Use Only. Not for Use in Diagnostic Procedures. Please consult the Safety Data Sheet for information regarding hazards and safe handling practices.

Storage/Stability

The kit is shipped on dry ice. Store all components at –20 °C upon receiving.

Preparation Instructions

Reagent Preparation

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

Sample Preparation
Tissue or cell samples (2×10^6) can be homogenized in 100 µL of phosphate buffered saline (PBS). Centrifuge at 14,000 rpm for 5 minutes. 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 filter). 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.

Procedures
Colorimetric Procedure
For the colorimetric assay, the linear detection range is is 4–400 µM citrate.

Standard Preparation
Prepare the 400 µM Citrate Standard Premix by mixing 10 µL of 10 mM Citrate Standard with 240 µL of ultrapure water. Next, dilute standards in 1.5-mL centrifuge tubes according to Table 1.
Note: If assaying culture medium with phenol red, dilute the Citrate Standard in culture medium.

Table 1.
Preparation of Citrate Standards

Tube	Standard Premix	Ultrapure Water	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

Reaction Mix
For each well of reaction, prepare Reaction Mix by mixing into a clean tube:
85 µL of Developer
1 µL of CL Enzyme Solution
1 µL of ODC Enzyme
1 µL of Dye Reagent

Sample Blank Reaction Mix
For each sample blank, prepare Sample Blank Reaction Mix by mixing into a clean tube:
85 µL of Developer
1 µL of ODC Enzyme
1 µL of Dye Reagent

Assay Reaction
1. Transfer 20 µL of standards into separate wells of a clear flat bottom 96 well plate.
2. Transfer 20 µL of each sample into separate wells of a clear flat bottom 96 well plate. Each sample being tested requires a sample blank.

   Note: If using an internal standard, samples will need three separate reactions:
   - Sample plus Internal Standard
   - Sample (alone)
   - Sample Blank

   For the internal standard prepare 500 µL of 1,000 µM Citrate Standard by mixing 50 µL of 10 mM Citrate Standard and 450 µL of ultrapure water.

   For the Sample plus Internal Standard well, add 5 µL of 1,000 µM citrate and 20 µL of sample.

   For the Sample and Sample Blank wells, add 5 µL of ultrapure water and 20 µL of sample.
3. Add 80 µL of the appropriate Reaction Mix to each Standard, Sample, and Sample Blank well. Mix well.
4. Incubate protected from light 15 minutes at room temperature.
5. Measure the absorbance at 570 nm (A_{570}).

Fluorometric Procedure
For the fluorometric assay, the linear detection range is is 1–40 µM citrate.
1. Dilute the Standards prepared in Colorimetric Procedure 10-fold in ultrapure water. If an internal standard is used, use 5 µL of 100 µM citrate.
2. Transfer 20 µL of standards and 20 µL of samples (prepare 2 wells per sample if a standard curve is used; prepare 3 wells per sample if an internal standard is used, see Colorimetric Procedure) into separate wells of a black 96 well plate.
3. Add 80 µL of appropriate Reaction Mix (see Colorimetric Procedure) to each well. Tap plate to mix.
4. Incubate protected from light for 15 minutes at room temperature.
5. Measure the fluorescence at λ_{ex} = 530 nm/ λ_{em} = 585 nm.

Results

Subtract the blank value (Standard Tube 4) from the standard values and plot the ΔA_{570} or ΔF against standard concentrations. Determine the slope using linear regression fitting. The citrate concentration of Sample is calculated as:

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

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

$$[\text{Citrate } (\mu M)] = \frac{R_{Sample} - R_{Blank}}{R_{Standard} - R_{Sample}} \times \frac{[\text{Standard}]}{4} \times n$$

where:

R_{Sample}, R_{Blank}, and $R_{Standard}$ = A_{570} or fluorescence readings of the Sample, Sample Blank, and the Sample plus Internal Standard, respectively.

n = the sample dilution factor.

Note: The volume of the internal standard is 4× lower than the sample volume; thus, the internal standard concentration should be divided by 4.

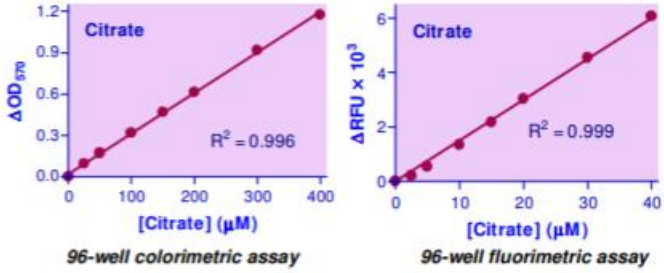
If the calculated citrate concentration is >400 µM for the colorimetric assay or >40 µM for the fluorimetric assay, dilute sample in ultrapure water and repeat assay. Multiply result by the dilution factor n.

Conversions

100 µM citrate = 19.1 mg/L, 0.0019%, or 19.1 ppm.

Figure 1.

Typical Standard Curves



References

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2. Costello, L.C., and O'Neill, J.J., A simplified and sensitive method for citrate determination in biological samples. J. Appl. Physiol., **27**, 120-2 (1969).
3. Parvin, R. et al., Convenient rapid determination of picomole amounts of oxaloacetate and citrate. Anal. Biochem., **104**, 296-9 (1980).

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