



Citrate Assay Kit

Citrate Assay Kit can be used to measure Citrate in serum, plasma, urine, Tissue/cell lysate and cell culture supernatants.

Catalog number: ARG81388

Package: 100 tests

For research use only. Not for use in diagnostic procedures.

TABLE OF CONTENTS

SECTION	Page
INTRODUCTION	3
PRINCIPLE OF THE ASSAY	4
MATERIALS PROVIDED & STORAGE INFORMATION.....	4
MATERIALS REQUIRED BUT NOT PROVIDED.....	5
TECHNICAL HINTS AND PRECAUTIONS	5
SAMPLE COLLECTION & STORAGE INFORMATION	5
REAGENT PREPARATION	7
ASSAY PROCEDURE	9
CALCULATION OF RESULTS.....	13
EXAMPLE OF TYPICAL STANDARD CURVE.....	14
QUALITY ASSURANCE	14

MANUFACTURED BY:

Arigo Biolaboratories Corporation

Address: 9F.-7, No. 12, Taiyuan 2nd St., Zhubei City,

Hsinchu County 302082, Taiwan

Tel: +886-3-6221320

Fax: +886-3-5530266

Email: info@arigobio.com

INTRODUCTION

Citric acid (Citric) is a weak organic acid that has the chemical formula $C_6H_8O_7$. It occurs naturally in citrus fruits. In biochemistry, Citrate is an intermediate in the TCA cycle (aka TriCarboxylic Acid cycle, or Krebs cycle, Szent-Györgyi), a central metabolic pathway for animals, plants, and bacteria. Citrate synthase catalyzes the condensation of oxaloacetate with acetyl CoA to form citrate. Citrate then acts as the substrate for aconitase and is converted into aconitic acid. The cycle ends with regeneration of oxaloacetate. This series of chemical reactions is the source of two-thirds of the food-derived energy in higher organisms. Hans Adolf Krebs received the 1953 Nobel Prize in Physiology or Medicine for the discovery.

Citrate can be transported out of the mitochondria and into the cytoplasm, then broken down into acetyl-CoA for fatty acid synthesis, and into oxaloacetate. High concentrations of cytosolic citrate can inhibit phosphofructokinase, the catalyst of a rate-limiting step of glycolysis. This effect is advantageous: high concentrations of citrate indicate that there is a large supply of biosynthetic precursor molecules, so there is no need for phosphofructokinase to continue to send molecules of its substrate, fructose 6-phosphate, into glycolysis. Citrate acts by augmenting the inhibitory effect of high concentrations of ATP, another sign that there is no need to carry out glycolysis. [Wikipedia: Citric acid]

PRINCIPLE OF THE ASSAY

This Citrate Activity Assay Kit provides a simple, and rapid procedure for measuring citrate concentration in serum, plasma, urine and cell culture supernatants samples. In this assay, citrate is converted to oxaloacetate and finally oxaloacetate is converted to pyruvate. The pyruvate is then oxidized with the conversion of the dye in the kit into a colored and fluorescent form. The color intensity at 570 nm or fluorescence intensity at λ ex/em = 530/585 nm is directly proportional to the citrate concentration in the sample. The concentration of citrate in the sample is then determined by comparing the signals of samples to the standard.

MATERIALS PROVIDED & STORAGE INFORMATION

Component	Quantity	Storage information
Citrate Developer	10 ml (Ready to use)	-20°C
Citrate Standard (10 mM)	500 μ l	-20°C
Citrate lyase Enzyme	1 vial (lyophilized)	-20°C
Oxaloacetate decarboxylase Enzyme	120 μ l	-20°C
Dye Reagent	120 μ l	-20°C

The kit is shipped on ice. Store all components at -20°C in dark. Shelf life of six months after receipt.

MATERIALS REQUIRED BUT NOT PROVIDED

- Microplate reader capable of measuring absorbance at 570 nm. Or fluorescence Microplate Reader capable of measuring fluorescence at $\lambda_{ex/em} = 530/585$ nm.
- Flat bottomed 96-well microplate or Black flat bottomed 96-well microplate
- Pipettes and pipette tips
- Deionized or distilled water.

TECHNICAL HINTS AND PRECAUTIONS

- Wear protective gloves, clothing, eye, and face protection especially while handling blood or body fluid samples.
- Prior to assay, concentrations of protein, inhibitor, substrate and incubation time may need to be established for a given sample.
- Briefly spin down the reagents before use.
- It is recommended that the standards and samples be assayed in at least duplicates.
- Change pipette tips between the addition of different reagent or samples.
- All reagents should be warmed to room temperature before use.

SAMPLE COLLECTION & STORAGE INFORMATION

The sample collection and storage conditions listed below are intended as general guidelines. Sample stability has not been evaluated.

Serum- Use a serum separator tube (SST) and allow samples to clot for 30 minutes before centrifugation for 15 minutes at 1000 x g at 2-8°C. Collect the

serum, serum should be deproteinated using a 10 kDa spin filter. Alternatively, the untreated serum can be measured directly if an internal standard is used. Aliquot and store samples at -80°C. Avoid repeated freeze-thaw cycles.

Plasma - Collect plasma using EDTA or heparin as an anticoagulant. Centrifuge for 15 minutes at 1000 x g at 2-8°C within 30 minutes of collection. Plasma samples should be deproteinated using a 10 kDa spin filter. Alternatively, the untreated plasma can be measured directly if an internal standard is used. Aliquot and store samples at -80°C. Avoid repeated freeze-thaw cycles.

Urine - Collect the urine by micturating directly into a sterile container. Remove impurities by centrifugation at 10,000 x g for 1 min. Collect the supernatants and assay immediately or aliquot and store samples at ≤-80°C.

It is recommended to dilute urine samples at least 5-fold and use internal standard method.

Tissue or cell samples - Homogenize 2 x 10⁶ of tissue or cells with 100 µL PBS. Then centrifuge at 10,000 rpm for 10 min. Collect the supernatants and assay immediately.

Cell Culture Supernatants - Media with high pyruvate concentrations should be avoided. (e.g. DMEM, L-15, F12, etc.).

Remove particulates by centrifugation for 10 min at 1500 x g at 4°C and aliquot & store samples at ≤-80°C. Avoid repeated freeze-thaw cycles.

Note: Samples should be clear and free of particles or precipitates. Avoid using haemolytic, icteric or lipaemic samples.

REAGENT PREPARATION

- **Citrate lyase Enzyme:** Add 120 μl of Citrate Developer to reconstitute the Citrate lyase Enzyme. Make sure the standard is dissolved completely. The reconstituted Citrate lyase Enzyme stock could be stored at -20°C for up to 4 weeks.

Before each use of the Citrate lyase Enzyme, please pipette or brief mix to assure the enzyme is resuspended.

- **Standard:**

For Colorimetric Procedure:

- Dilute 10 mM Citrate Standard solutions with deionized water to yield 400 μM , 240 μM and 120 μM standard as following table, the deionized water serves as zero standard (0 μM). If assaying culture media with phenol red, dilute the Citrate Standard in culture media instate of deionized water.

The example of the dilution of standards

Standard No.	Standard Conc. μM	Deionized water (culture media) (μl)	Standard (μl)
S1	400	240	10 μl of 10 mM Stock
S2	240	40	60 μl of S1
S3	120	70	30 μl of S1
S0	0	100	0

For fluorimetric Procedure:

- The linear range of ARG81388 is 0.5 to 40 μM citrate. Dilute the standards prepared in Colorimetric Procedure with deionized water (or culture media) at 1:10 dilution to yield standard concentration as 40 μM , 24 μM and 12 μM .

- **Working Reagent:**

For each reaction combine the following (*Prepare before use*):

- 85 µL of Citrate Developer
- 1 µL of Citrate lyase Enzyme
- 1 µL of Oxaloacetate decarboxylase Enzyme
- 1 µL Dye Reagent.

- **Blank Working Reagent:**

For each reaction combine the following (*Prepare before use*):

- 86 µL of Citrate Developer
- **(without Citrate lyase Enzyme)**
- 1 µL of Oxaloacetate decarboxylase Enzyme
- 1 µL Dye Reagent.

- **Citrate Developer:** Citrate Developer is ready to use, mix it well by vigorous shaking before use.

ASSAY PROCEDURE

All materials should be equilibrated to room temperature (RT) before use, each vial should be mixed thoroughly without foaming and briefly centrifuge tubes prior to use. Each sample requires a sample blank.

For Colorimetric Procedure:

1. Add **20 µL** of **each standard (S0-S3)** to standard wells in a 96 well plate.
2. Add **20 µl** of **sample** in sample well, and sample blank well.
3. Add **80 µL** of the **Working Reagent** to each standard well and sample well.
4. Add **80 µL** of the **Blank Working Reagent** to sample blank wells.
5. Gently tap the plate to ensure thorough mixing. Incubate for **15 min** at **room temperature** in dark.
6. Gently tap the plate to ensure thorough mixing. Incubate for **15 min** at **room temperature** in dark.
7. Read the OD with a microplate reader at **570 nm**.

Reagent	Standard S0-S3	Sample	Sample Blank
Sample		20 µl	20 µl
Standard	20 µl		
Working Reagent	80 µl	80 µl	
Blank Working Reagent			80 µl
Mix well and incubate for 15 min at RT in dark.			
Read the OD with a microplate reader at 570nm .			

Note: The concentration of Standard S1-S3: 400 µM, 240 µM, 120 µM for Colorimetric Procedure. Please refer the detail at REAGENT PREPARATION section.

Citrate Assay Kit ARG81388

- 1.1 For un-filtered serum, plasma and urine samples, it is recommended to used internal standard method.
- 1.2 Diluted the 10 M citrate with ddH₂O to yield 1000 μ M citrate as internal standard. (ex. 50 μ L 10 mM Standard and 450 μ L ddH₂O)
1. Add **20 μ L** of **sample** in internal standard well, sample well, and sample blank well.
 2. Add **5 μ L** of **1000 μ M citrate (internal standard)** in internal standard well.
Add **5 μ L** of **ddH₂O** in sample well and sample blank well.
 3. Add **80 μ L** of the **Working Reagent** to internal standard well, and sample well.
 4. Add **80 μ L** of the **Blank Working Reagent** to sample blank wells.
 5. Gently tap the plate to ensure thorough mixing. Incubate for **15 min** at **room temperature** in dark.
 6. Read the OD with a microplate reader at **570 nm**.

Reagent	Internal Standard	Sample	Sample Blank
Sample	20 μ L	20 μ L	20 μ L
1000 μ M citrate	5 μ L		
ddH ₂ O		5 μ L	5 μ L
Working Reagent	80 μ L	80 μ L	
Blank Working Reagent			80 μ L
Mix well and incubate for 15 min at RT in dark.			
Read the OD with a microplate reader at 570nm .			

Note: If the internal standard is used in the assay the regular standard (S0-S3) are not necessary for the assay. The concentration of Internal Standard is 1000 μ M citrate for Colorimetric Procedure. Please refer the detail at REAGENT PREPARATION section.

Citrate Assay Kit ARG81388

For Fluorimetric Procedure (black 96 well plate is used):

1. Add **20 µL** of **each standard (S0-S3)** to separate wells in a 96 well plate.
2. Add **20 µl** of **sample** in sample well, and sample blank well.
3. Add **80 µL** of the **Working Reagent** to each standard well and sample well.
4. Add **80 µL** of the **Blank Working Reagent** to sample blank wells.
5. Gently tap the plate to ensure thorough mixing. Incubate for **15 min** at **room temperature** in dark.
6. Read fluorescence intensity at $\lambda_{ex} = 530 \text{ nm}$ and $\lambda_{em} = 585 \text{ nm}$.

Reagent	Standard S0-S3	Sample	Sample Blank
Sample		20 µl	20 µl
Standard	20 µl		
Working Reagent	80 µl	80 µl	
Blank Working Reagent			80 µl
Mix well and incubate for 15 min at RT in dark.			
Read fluorescence intensity at $\lambda_{ex/em} = 530 / 585 \text{ nm}$.			

Note: The concentration of Standard S1-S3: 40 µM, 24 µM, 12 µM for Fluorimetric Procedure. Please refer the detail at REAGENT PREPARATION section.

Citrate Assay Kit ARG81388

2.1 For un-filtered serum, plasma and urine samples, it is recommended to used internal standard method.

2.2 Diluted the 10 M citrate with ddH₂O to yield 100 μ M citrate as internal standard.

1. Add 20 μ l of **sample** in internal standard well, sample well, and sample blank well.
2. Add 5 μ l of **100 μ M citrate (internal standard)** in internal standard well.
Add 5 μ l of **ddH₂O** in sample well and sample blank well.
3. Add 80 μ l of the **Working Reagent** to internal standard well, and sample well.
4. Add 80 μ l of the **Blank Working Reagent** to sample blank wells.
5. Gently tap the plate to ensure thorough mixing. Incubate for **15 min** at **room temperature** in dark.
6. Read fluorescence intensity at $\lambda_{ex} = 530 \text{ nm}$ and $\lambda_{em} = 585 \text{ nm}$.

Reagent	Internal Standard	Sample	Sample Blank
Sample	20 μ l	20 μ l	20 μ l
100 μ M citrate (internal standard)	5 μ l		
ddH ₂ O		5 μ l	5 μ l
Working Reagent	80 μ l	80 μ l	
Blank Working Reagent			80 μ l
Mix well and incubate for 15 min at RT in dark.			
Read fluorescence intensity at $\lambda_{ex/em} = 530 / 585 \text{ nm}$.			

Note: If the internal standard is used in the assay the regular standard (S0-S3) are not necessary for the assay. The concentration of Internal Standard is 100 μ M citrate for Fluorimetric Procedure.

Please refer the detail at REAGENT PREPARATION section.

CALCULATION OF RESULTS

1. If S0-S3 are assayed in the kit. Subtract the blank value (S0) 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:

[Citrate] (μM) =

$$N \times [(R_{\text{SAMPLE}} - R_{\text{BLANK}}) / \text{Slope } (\mu M^{-1})]$$

2. If use internal standard, the sample citrate concentration is computed as follows:

[Citrate] (μM) =

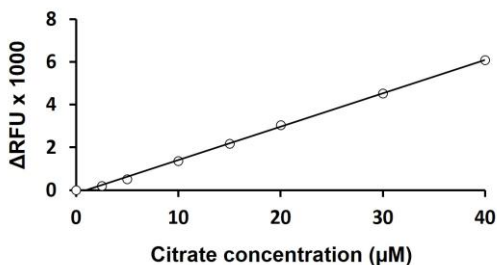
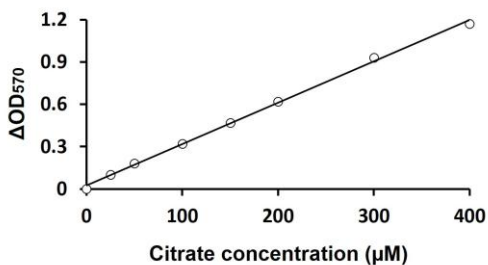
$$N \times [(R_{\text{Sample}} - R_{\text{Blank}}) / (R_{\text{Standard}} - R_{\text{Sample}})] \times (\text{Standard Conc.} / 4)$$

3. The R_{Sample} , R_{Blank} and R_{Standard} are OD or fluorescence readings of the Sample, Sample Blank and the Sample plus Internal Standard respectively. N is the sample dilution factor. **Standard Conc.** is the concentration of internal standard (1000 μM citrate for Colorimetric Procedure, 100 μM citrate for Fluorimetric Procedure).

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

4. If the calculated citrate concentration is $>400 \mu M$ for the colorimetric assay, or $>40 \mu M$ for the fluorimetric assay, dilute sample in deionized water and repeat assay. Multiply result by the dilution factor N .
5. Conversions: The 100 μM citrate equals 19.1 mg/L, 0.0019% or 19.1 ppm.

EXAMPLE OF ASSAY



QUALITY ASSURANCE

Sensitivity

Linear detection range:

Colorimetric assays: 4 to 400 μM

Fluorimetric assays: 0.5 to 40 μM

The minimum detectable dose (MDD) of Citrate:

Colorimetric assays: 4 μM

Fluorimetric assays: 0.5 μM