ab83396

Citrate Assay Kit (Colorimetric/Fluorometric)

Instructions for Use

For the rapid, sensitive and accurate measurement of Citrate levels in various samples.

View kit datasheet: www.abcam.com/ab83396
(use www.abcam.cn/ab83396 for China, or www.abcam.co.jp/ab83396 for Japan)

This product is for research use only and is not intended for diagnostic use.

PLEASE NOTE: With the acquisition of BioVision by Abcam, we have made some changes to component names and packaging to better align with our global standards as we work towards environmental-friendly and efficient growth. You are receiving the same high-quality products as always, with no changes to specifications or protocols.

Version 8 Last Updated 13 April 2023

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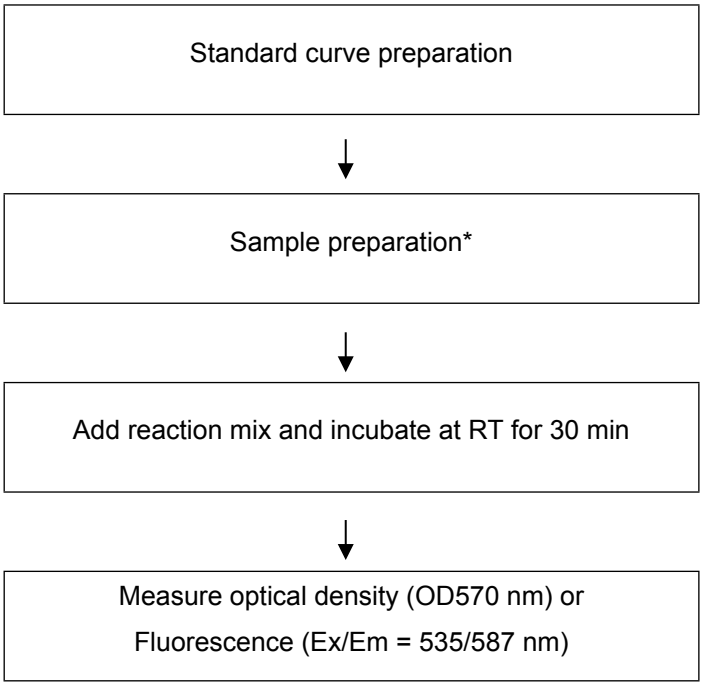
1. BACKGROUND

Citrate Assay Kit (Colorimetric/Fluorometric) (ab83396) provides a simple, sensitive and rapid means of quantifying citrate in a variety of samples. In the assay, citrate is converted to pyruvate via oxaloacetate. The pyruvate is quantified by converting a nearly colorless probe to an intensely colored (570 nm) and fluorescent (Ex/Em: 535/587 nm) product. The Citrate Assay Kit can detect 0.1 to 10 nmoles (~2 µM-10 mM) of citrate in a variety of samples.

Citric acid (HOOC-CH_2-C(-OH)(-COOH)-CH_2-COOH) is a key intermediate in the TCA cycle which occurs in mitochondria. It is formed by the addition of oxaloacetate to the acetyl group of acetyl-CoA derived from the glycolytic pathway. Citrate can be transported out of mitochondria and converted back to acetyl CoA for fatty acid synthesis. Citrate is an allosteric modulator of both fatty acid synthesis (acetyl-CoA carboxylase) and glycolysis (phospho-fructokinase). Citrate is widely used industrially in foods, beverages and pharmaceuticals. Citrate metabolism and disposition can vary widely due to sex, age and a variety of other factors.

2. ASSAY SUMMARY



*Samples may require deproteinization.

3. PRECAUTIONS

Please read these instructions carefully prior to beginning the assay.

All kit components have been formulated and quality control tested to function successfully as a kit. Modifications to the kit components or procedures may result in loss of performance.

4. STORAGE AND STABILITY

Store kit at -20ºC in the dark immediately upon receipt. Kit has a storage time of 1 year from receipt, providing components have not been reconstituted.

Refer to list of materials supplied for storage conditions of individual components. Observe the storage conditions for individual prepared components in section 5.

Aliquot components in working volumes before storing at the recommended temperature. **Reconstituted components are stable for 2 months.**

5. MATERIALS SUPPLIED

Item	Amount	Storage Condition (Before Preparation)	Storage Condition (After Preparation)
Citrate Assay Buffer	25 mL	-20°C	-20°C
OxiRed Probe/Citrate Probe	0.2 mL	-20°C	-20°C
Citrate Enzyme Mix (Lyophilized)	1 vial	-20°C	-20°C
Citrate Developer (Lyophilized)	1 vial	-20°C	-20°C
Citrate Standard (10 µmol; Lyophilized)	1 vial	-20°C	-20°C

6. MATERIALS REQUIRED, NOT SUPPLIED

These materials are not included in the kit, but will be required to successfully perform this assay:

- MilliQ water or other type of double distilled water (ddH_2O)
- PBS
- Microcentrifuge
- Pipettes and pipette tips
- Colorimetric or fluorescent microplate reader – equipped with filter for OD570 nm or Ex/Em = 535/587 nm (respectively)
- 96 well plate: clear plates for colorimetric assay; black plates (clear bottoms) for fluorometric assay
- Heat block or water bath
- Vortex
- Dounce homogenizer or pestle (if using tissue)

For deproteinization step, additional reagents are required:

- Perchloric acid (PCA) 4M, ice cold
- Potassium Hydroxide (KOH) 2M
- 10 kD Spin Columns (ab93349) – for fluid samples, if not performing PCA precipitation

7. LIMITATIONS

- Assay kit intended for research use only. Not for use in diagnostic procedures.
- Do not use kit or components if it has exceeded the expiration date on the kit labels.
- Do not mix or substitute reagents or materials from other kit lots or vendors. Kits are QC tested as a set of components and performance cannot be guaranteed if utilized separately or substituted.

8. TECHNICAL HINTS

- **This kit is sold based on number of tests. A 'test' simply refers to a single assay well. The number of wells that contain sample, control or standard will vary by product. Review the protocol completely to confirm this kit meets your requirements. Please contact our Technical Support staff with any questions.**
- Keep enzymes, heat labile components and samples on ice during the assay.
- Make sure all buffers and solutions are at room temperature before starting the experiment.
- Samples generating values higher than the highest standard should be further diluted in the appropriate sample dilution buffers.
- Avoid foaming or bubbles when mixing or reconstituting components.
- Avoid cross contamination of samples or reagents by changing tips between sample, standard and reagent additions.
- Ensure plates are properly sealed or covered during incubation steps.
- Ensure complete removal of all solutions and buffers from tubes or plates during wash steps.
- Make sure you have the right type of plate for your detection method of choice.
- Make sure the heat block/water bath and microplate reader are switched on.

9. REAGENT PREPARATION

- Briefly centrifuge small vials at low speed prior to opening.

9.1 **Citrate Assay Buffer:**

Ready to use as supplied. Equilibrate to room temperature before use. Store at - 20°C.

9.2 **Citrate Standard:**

Reconstitute the Citrate Standard (10 µmol) in 100 µL of ddH_2O to generate a 100 mM standard stock solution. Keep on ice while in use. Aliquot standard so that you have enough to perform the desired number of assays.

Store at - 20°C.

9.3 **OxiRed Probe/Citrate Probe (in DMSO):**

Ready to use as supplied. Warm by placing in a 37°C bath for 1 – 5 minutes to thaw the DMSO solution before use. **NOTE: DMSO tends to be solid when stored at -20°C, even when let at room temperature, so it needs to melt for few minutes at 37°C.** Aliquot probe so that you have enough volume to perform the desired number of assays. Store at - 20°C protected from light. Once the probe is thawed, use with two months.

9.4 **Citrate Developer:**

Dissolve in 220 µL Assay Buffer. Keep on ice during the assay. Aliquot developer so that you have enough volume to perform the desired number of assays. Store at -20ºC. Avoid repeated freeze/thaw cycles. Use within two months.

9.5 **Citrate Enzyme Mix:**

Dissolve in 220 µL Assay Buffer. Keep on ice during the assay. Aliquot enzyme mix so that you have enough volume to perform the desired number of assays. Store at -20ºC. Avoid repeated freeze/thaw cycles. Use within two months.

10. STANDARD PREPARATION

- Always prepare a fresh set of standards for every use.
- Diluted standard solution is unstable and must be used within 4 hours.

10.1 **For the colorimetric assay:**

10.1.1 Prepare 0.5 mL of 1 nmol/µL standard by adding 5 µL of the 100 nmol/µL standard into 495 µL of H_2O.

10.1.2 Using 1 nmol/µL standard, prepare standard curve dilution as described in the table in a microplate or microcentrifuge tubes:

Standard #	Volume of Standard (µL)	Assay Buffer (µL)	Final volume standard in well (µL)	End [Citrate] in well
1	0	150	50	0 nmol/well
2	6	144	50	2 nmol/well
3	12	138	50	4 nmol/well
4	18	132	50	6 nmol/well
5	24	126	50	8 nmol/well
6	30	120	50	10 nmol/well

Each dilution has enough amount of standard to set up duplicate readings (2 x 50 µL).

10.2 **For the flurometric assay:**

10.2.1 Prepare a 1 nmol/µL standard by following step 10.1.1.

10.2.2 Prepare 100 µL of 0.1 nmol/µL by diluting 10 µL of 1 nmol/µL Standard to 90 µL of dH_2O.

10.2.3 Using 0.1 nmol/µL standard, prepare standard curve dilution as described in the table in a microplate or microcentrifuge tubes:

Standard #	Volume of Standard (µL)	Assay Buffer (µL)	Final volume standard in well (µL)	End [Citrate] in well
1	0	150	50	0 nmol/well
2	6	144	50	0.2 nmol/well
3	12	138	50	0.4 nmol/well
4	18	132	50	0.6 nmol/well
5	24	126	50	0.8 nmol/well
6	30	120	50	1.0 nmol/well

Each dilution has enough amount of standard to set up duplicate readings (2 x 50 µL).

NOTE: *If your sample readings fall out the range of your fluorometric standard curve, you might need to adjust the dilutions and create a new standard curve.*

11. SAMPLE PREPARATION

General Sample information:

- We recommend performing several dilutions of your sample to ensure the readings are within the standard value range.
- We recommend that you use fresh samples. If you cannot perform the assay at the same time, we suggest that you complete the Sample Preparation step as well as the Deproteinization step before storing the samples. Alternatively, if that is not possible, we suggest that you snap freeze cells or tissue in liquid nitrogen upon extraction and store the samples immediately at -80°C. When you are ready to test your samples, thaw them on ice. Be aware however that this might affect the stability of your samples and the readings can be lower than expected.

11.1 Cell (adherent or suspension) samples:

11.1.1 Harvest the amount of cells necessary for each assay (initial recommendation = 2 x 10^6 cells).

11.1.2 Wash cells with cold PBS.

11.1.3 Resuspend cells in 100 µL of Citrate Assay Buffer/Assay Buffer.

11.1.4 Homogenize cells quickly by pipetting up and down a few times.

11.1.5 Centrifuge sample for 2 – 5 minutes at 4°C at top speed using a cold microcentrifuge to remove any insoluble material.

11.1.6 Collect supernatant and transfer to a clean tube.

11.1.7 Keep on ice.

11.1.8 Perform deproteinization step as described in section 11.5.

11.2 Tissue samples:

11.2.1 Harvest the amount of tissue necessary for each assay (initial recommendation = 20 mg tissue).

11.2.2 Wash tissue in cold PBS.

11.2.3 Resuspend tissue in 100 µL of Citrate Assay Buffer/Assay Buffer.

11.2.4 Homogenize tissue with a Dounce homogenizer sitting on ice, with 10 – 15 passes.

11.2.5 Centrifuge samples for 2 – 5 minutes at 4°C at top speed using a cold microcentrifuge to remove any insoluble material.

11.2.6 Collect supernatant and transfer to a clean tube.

11.2.7 Keep on ice.

11.2.8 Perform deproteinization step as described in section 11.5.

11.3 **Plasma, Serum and Urine:**

Plasma, serum and urine samples generally contain high amount of proteins, so they should be deproteinized as described in section 11.5.

Alternatively, you can use 10kD Spin column (ab93349) to deproteinize biological fluids.

11.4 **Other biological samples (Cell culture media):**

Cell culture media samples can be directly used. It is recommended to spin down for 1-2 minutes at top speed on a microcentrifuge to ensure there are no debris/cells floating in the medium.

11.5 **Deproteinization step:**

Prepare samples as specified in protocol. You should have a clear protein sample after homogenization and centrifugation. Keep your samples on ice.

11.5.1 Add ice cold PCA 4 M to a final concentration of 1 M in the homogenate solution and vortex briefly to mix well. ***NOTE****: high protein concentration samples might need more PCA.*

11.5.2 Incubate on ice for 5 minutes.

11.5.3 Centrifuge samples at 13,000 x g for 2 minutes at 4°C in a cold centrifuge and transfer supernatant to a fresh tube. Measure volume of supernatant.

11.5.4 Precipitate excess PCA by adding ice-cold 2M KOH that equals 34% of the supernatant to your sample (for instance, 34 µL of 2 M KOH to 100 µL sample) and vortexing briefly. This will neutralize the sample and precipitate excess PCA.

11.5.5 After neutralization, it is very important that pH equals 6.5 – 8 (use pH paper to test 1 µL of sample). If necessary, adjust pH with 0.1 M KOH or PCA.

11.5.6 Centrifuge at 13,000 x g for 15 minutes at 4°C and collect supernatant.

Samples are now deproteinized, neutralized and PCA has been removed. The samples are now ready to use in the assay.

Sample Recovery

The deproteinized samples will be diluted from the original concentration.

To calculate the dilution factor of your final sample, simply apply the following formula:

$$\% \text{ original concentration} = \frac{\text{Initial sample volume}}{\text{(initial sample volume + vol PCA + vol KOH)}} \text{ X100}$$

NOTE: *We suggest using different volumes of sample to ensure readings are within the Standard Curve range.*

12. ASSAY PROCEDURE and DETECTION

- **Equilibrate all materials and prepared reagents to room temperature prior to use.**
- **It is recommended to assay all standards, controls and samples in duplicate.**

12.1 **Set up Reaction wells:**

- Standard wells = 50 µL standard dilutions.
- Sample wells = 1 – 50 µL samples (adjust volume to 50 µL/well with Citrate Assay Buffer/Assay Buffer).
- Background control sample wells = 1 – 50 µL samples (adjust volume to 50 µL/well with Citrate Assay Buffer/Assay Buffer). ***NOTE:*** *for samples containing oxaloacetate or pyruvate, as they can generate background.*

12.2 **Citrate Reaction Mix (COLORIMETRIC ASSAY):**

Prepare 50 µL of Reaction Mix for each reaction:

Component	Reaction Mix (µL)	Background Reaction Mix (µL)
Citrate Assay Buffer/Assay Buffer	**44**	**46**
Citrate Enzyme Mix/Enzyme Mix	**2**	**0**
Citrate Developer/Developer	**2**	**2**
OxiRed Probe/Citrate Probe	**2**	**2**

Mix enough reagents for the number of assays (samples, standards and background control) to be performed. Prepare a master mix of the Reaction Mix to ensure consistency. We recommend the following calculation:

X µL component x (Number samples + Standards +1)

12.3 **Citrate Reaction Mix (FLUOROMETRIC ASSAY):**

Dilute OxiRed Probe/Citrate Probe 10X in DMSO

Component	Reaction Mix (µL)	Background Reaction Mix (µL)
Citrate Assay Buffer/Assay Buffer	**44**	**46**
Citrate Enzyme Mix/Enzyme Mix	**2**	**0**
Citrate Developer/Developer	**2**	**2**
OxiRed Probe/Citrate Probe*	**2**	**2**

**For fluorometric readings, using OxiRed Probe/Citrate Probe diluted 10X decreases the fluorescent background readings, therefore increasing detection sensitivity.*

Mix enough reagents for the number of assays (samples, standards and background control) to be performed. Prepare a master mix of the Reaction Mix to ensure consistency. We recommend the following calculation:

X µL component x (Number samples + Standards +1)

12.4 Add 50 µL of Reaction Mix into each standard and sample well.

12.5 Add 50 µL of Background Reaction Mix into background sample control well.

12.6 Incubate at room temperature for 30 min protected from light.

12.7 Measure output on a microplate reader:

- Colorimetric assay: measure OD570 nm.
- Fluorometric assay: measure Ex/Em = 535/587 nm.

13. CALCULATIONS

- Samples producing signals greater than that of the highest standard should be further diluted in appropriate buffer and reanalyzed, then multiplying the concentration found by the appropriate dilution factor.
- For statistical reasons, we recommend each sample should be assayed with a minimum of two replicates (duplicates).

13.1 Average the duplicate reading for each standard and sample.

13.2 If the sample background control is significant, then subtract the sample background control from sample reading.

13.3 Subtract the mean absorbance value of the blank (Standard #1) from all standard and sample readings. This is the corrected absorbance.

13.4 Plot the corrected absorbance values for each standard as a function of the final concentration of citrate.

13.5 Draw the best smooth curve through these points to construct the standard curve. Most plate reader software or Excel can plot these values and curve fit. Calculate the trendline equation based on your standard curve data (use the equation that provides the most accurate fit).

13.6 Extrapolate sample readings from the standard curve plotted using the following equation:

$$Ay = \left(\frac{Corrected\ absorbance - (y - intercept)}{Slope}\right)$$

13.7 Concentration of samples in the test samples is calculated as:

$$Concentration = \left(\frac{Ay}{Sv}\right) * D$$ = nmol/µL = µmol/ml = mM

Where:

Ay = Amount of citrate in the sample well (nmol).

Sv = Sample volume added into the reaction well (µL).

D = Sample dilution factor.

Citric acid molecular weight: 191 g/mol

14. TYPICAL DATA

TYPICAL STANDARD CURVE – Data provided for **demonstration purposes only**. A new standard curve must be generated for each assay performed.

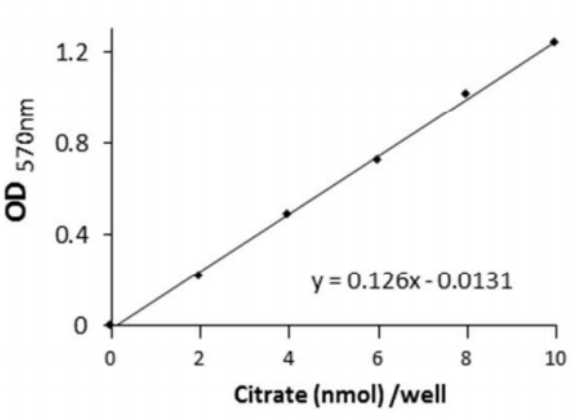


Figure 1. Typical Citrate Standard calibration curve using colorimetric reading.

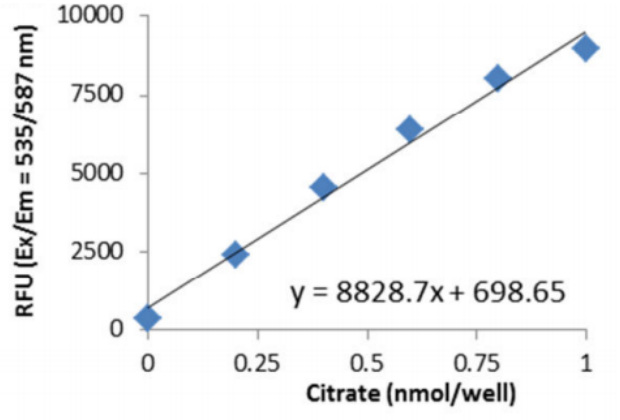


Figure 2. Typical Citrate Standard calibration curve using fluorometric reading.

15. QUICK ASSAY PROCEDURE

***NOTE**: This procedure is provided as a quick reference for experienced users. Follow the detailed procedure when performing the assay for the first time:*

- Prepare standard, thaw OxiRed Probe/Citrate probe and prepare enzyme mix (aliquot if necessary); get equipment ready.
- Prepare appropriate standard curve for your detection method of choice (colorimetric or fluorometric).
- Prepare samples in duplicate (find optimal dilutions to fit standard curve readings).
- Set up plate for standard (50 µL), samples (50 µL) and background wells (50 µL).
- Prepare Citrate Reaction Mix (Number samples+standards+1)

Component	Colorimetric Reaction Mix (µL)	Color Background Reaction Mix (µL)
Citrate Assay Buffer/Assay	44	46
Citrate Enzyme Mix/Enzyme Mix	2	0
Citrate Developer/ Developer	2	2
OxiRed Probe/Citrate Probe	2	2

Component	Fluorometric Reaction Mix (µL)	Fluor Background Reaction Mix (µL)
Citrate Assay Buffer/Assay Buffer	44	46
Citrate Enzyme Mix/Enzyme Mix	2	0
Citrate Developer/ Developer	2	2
OxiRed Probe/Citrate Probe* (1/10)	2	2

- Add 50 µL Reaction Mix / Background Reaction Mix to respective wells.
- Incubate plate at RT for 30min.
- Measure plate at OD570 nm for colorimetric assay or Ex/Em= 535/587 nm for fluorometric assay.

16. TROUBLESHOOTING

Problem	Cause	Solution
Assay not working	Use of ice-cold buffer	Buffers must be at room temperature
	Plate read at incorrect wavelength	Check the wavelength and filter settings of instrument
	Use of a different 96-well plate	Colorimetric: Clear plates Fluorometric: black wells/clear bottom plate
Sample with erratic readings	Samples not deproteinized (if indicated on protocol)	Use PCA precipitation protocol for deproteinization
	Cells/tissue samples not homogenized completely	Use Dounce homogenizer, increase number of strokes
	Samples used after multiple free/ thaw cycles	Aliquot and freeze samples if needed to use multiple times
	Use of old or inappropriately stored samples	Use fresh samples or store at -80°C (after snap freeze in liquid nitrogen) till use
	Presence of interfering substance in the sample	Check protocol for interfering substances; deproteinize samples
Lower/ Higher readings in samples and Standards	Improperly thawed components	Thaw all components completely and mix gently before use
	Allowing reagents to sit for extended times on ice	Always thaw and prepare fresh reaction mix before use
	Incorrect incubation times or temperatures	Verify correct incubation times and temperatures in protocol

Problem	Cause	Solution
Standard readings do not follow a linear pattern	Pipetting errors in standard or reaction mix	Avoid pipetting small volumes (< 5 µL) and prepare a master mix whenever possible
	Air bubbles formed in well	Pipette gently against the wall of the tubes
	Standard stock is at incorrect concentration	Always refer to dilutions on protocol
Unanticipated results	Measured at incorrect wavelength	Check equipment and filter setting
	Samples contain interfering substances	Troubleshoot if it interferes with the kit
	Sample readings above/ below the linear range	Concentrate/ Dilute sample so it is within the linear range

17. FAQ

There is a lot of variation from one standard curve to another with the same kit. Will preparing a master mix for the reactions help minimize this? Do you recommend pipetting up and down or using plate shaker?
We definitely recommend preparing master mixes for both reaction mix and background controls. This minimizes chances of pipetting errors from pipetting small volumes for each sample. We recommend using a plate shaker but shake gently so that there is no mixing between wells. Pipetting up/down is also possible but extreme care needs to be taken not to cross contaminate between wells.

If starting with one million (1 X 10^6) cells, what sample dilution is recommended for the assay?
The optimum dilution factor depends on the cell type, the concentration of citrate in the cells and the overall quality of the lysate. It is recommended making a series of dilutions over a broad range (the datasheet recommends 1-50 µL per well) to make sure you get the best results which will read within the linear range of the standard curve.

What is the detection range of the assay?
This kit can detect 0.1 to 10 nmoles (~2 µM-10 mM) of citrate in a variety of samples.

Can sodium citrate be detected by this kit?
It can detect sodium citrate.

What are the incompatible sample types for this kit?
Incompatible samples can be any sample like blood collected with citrate as the anti-coagulant or medium with added citrate in it. This will likely cause saturation and skew the results due to the citrate concentrations being greater than the range (2uM-10mM).

18. INTERFERENCES

These chemicals or biological materials will cause interferences in this assay causing compromised results or complete failure:

- Anti-coagulant or medium containing citrate will cause interfere with this assay.
- Calcium bound-citrate in solution has to be released so the enzyme in the kit can use the free citrate to catalyze the first detection reaction.

19. **NOTES**

For all technical and commercial enquires please go to:
www.abcam.com/contactus
www.abcam.cn/contactus (China)
www.abcam.co.jp/contactus (Japan)