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ab239712
Citrate Synthase Assay Kit

For the measurement of citrate synthase activity in various tissues/cells.

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

Table of Contents

1. Overview 1
2. Protocol Summary 2
3. General guidelines, precautions, and troubleshooting 3
4. Materials Supplied, and Storage and Stability 4
5. Materials Required, Not Supplied 4
6. Reagent Preparation 5
7. Standard Preparation 6
8. Sample Preparation 7
9. Assay Procedure 8
10. Data Analysis 9
11. Typical Data 10
13. Notes 11

1. Overview

Citrate Synthase Assay Kit (ab239712) provides reagents for the measurement of citrate synthase activity in various tissues/cells. Citrate Synthase reacts with substrate mix to form an intermediate, which subsequently reacts with developer to generate the colored product. The rate of color development is proportional to the enzyme activity. The assay is simple, rapid and can detect Citrate Synthase activity less than 1 mU in a variety of samples.

2. Protocol Summary

Prepare samples

Prepare GSH Standard curve.

Prepare Reaction Mix and add 50 µL of Reaction Mix to each well containing samples, Positive Control and Standards. Add 50 µL of Background Control Mix into sample background control well(s). Mix well.

Measure absorbance (OD 412 nm) immediately in kinetic mode at 25°C for 20-40 mins.

3. General guidelines, precautions, and troubleshooting

- Please observe safe laboratory practice and consult the safety datasheet.
- For general guidelines, precautions, limitations on the use of our assay kits and general assay troubleshooting tips, particularly for first time users, please consult our guide: www.abcam.com/assaykitguidelines
- For typical data produced using the assay, please see the assay kit datasheet on our website.

4. Materials Supplied, and Storage and Stability

- Store kit at -20°C in the dark immediately upon receipt and check below in Section 6 for storage for individual components. Kit can be stored for 1 year from receipt, if components have not been reconstituted.
- Aliquot components in working volumes before storing at the recommended temperature.

Item	Quantity	Storage condition
CS Assay Buffer	25 mL	-20°C
CS Substrate Mix (Lyophilized)	1 vial	-20°C
CS Developer (Lyophilized)	1 vial	-20°C
GSH Standard (reduced) (Lyophilized)	1 vial	-20°C
CS Positive Control (Lyophilized)	1 vial	-20°C

5. Materials Required, Not Supplied

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

- 96-well plate with flat bottom.
- Multi-well spectrophotometer

6. Reagent Preparation

- Before using the kit, spin tubes and bring down all components to the bottom of tubes.
- Prepare only as much reagent as is needed on the day of the experiment.

6.1 CS Assay Buffer:

Store at -20°C. Bring to room temperature (RT) before use.

6.2 CS Substrate Mix:

Reconstitute with 220 µL dH_2O. Aliquot and store at -20°C. Keep on ice while in use. Use within two months.

6.3 CS Developer:

Reconstitute with 1 mL CS Assay Buffer. Pipette up and down to dissolve completely. Aliquot and store at -20°C. Keep on ice while in use. Use within two months.

6.4 GSH Standard (reduced):

Reconstitute with 100 µL dH_2O to make 20 mM GSH Standard solution. Aliquot and store at -20°C. Keep on ice while in use. Use within two months.

6.5 CS Positive Control:

Reconstitute with 100 µL CS Assay Buffer to make the stock solution and mix thoroughly. Aliquot and store at 20°C. Keep on ice while in use. Use within two months.

7. Standard Preparation

- Always prepare a fresh set of standards for every use.
- Discard working standard dilutions after use as they do not store well.

7.1 Dilute GSH Standard to 2 mM by adding 10 µL of 20 mM Standard to 90 µL of Assay Buffer.

7.2 Add 0, 4, 8, 12, 16, 20 µL of diluted GSH Standard into 96-well plate and adjust the volume to 50 µL with Assay Buffer to generate 0, 8, 16, 24, 32 and 40 nmol GSH Standard/well.

Standard #	2mM GSH Standard (µL)	Assay Buffer (µL)	GSH/well
1	0	50	0 nmol
2	4	46	8 nmol
3	8	42	16 nmol
4	12	38	24 nmol
5	16	34	32 nmol
6	20	30	40 nmol

8. Sample Preparation

8.1 Homogenize tissue (10 mg) or cells (1 x 10^6) on ice with 100 µL ice cold CS Assay Buffer. Keep on ice for 10 mins.

8.2 Centrifuge at 10,000 x *g* for 5 mins. Collect the supernatant.

8.3 Add 1-50 µL sample into a 96-well plate. Adjust the volume to 50 µL with CS Assay Buffer.

8.4 Alternatively, isolate mitochondria from fresh tissues or cells. Add 1-50 µL of isolated mitochondrial sample into a 96-well plate and adjust the volume to 50 µl with CS Assay Buffer.

Δ Note:

- For unknown samples, we suggest testing several doses to ensure the readings are within the Standard Curve range.
- For samples having high CoA level, prepare parallel sample well(s) as background control.

9. Assay Procedure

9.1 Dilute CS Positive Control 100 times by adding 10 µL of stock solution into 990 µL of CS Assay Buffer. Add 2-20 µL of diluted CS Positive Control into desired well(s) and adjust the volume to 50 µL with CS Assay Buffer.

9.2 Prepare 50 µL Reaction Mix for each well to be assayed as per the table and mix well. Add 50 µL of Reaction Mix into Standard, Positive Control and sample wells. Mix well.

	Reaction Mix	Background Control Mix
CS Assay Buffer	43 µL	45 µL
CS Developer	5 µL	5 µL
CS Substrate Mix	2 µL	-

Δ Note: For background correction, add Background Control Mix to background control well(s) and mix well.

9.3 Measure absorbance (OD 412 nm) immediately in kinetic mode at 25°C for 20-40 mins.

Δ Note: Incubation time depends on the Citrate Synthase activity in the samples. We recommend measuring the OD in a kinetic mode, and choosing two time points (T1 and T2) in the linear range to calculate the Citrate Synthase Activity of the samples.

10. Data Analysis

10.1 Subtract 0 Standard reading from all readings. Plot the GSH Standard Curve.

10.2 If sample background control reading is significant then subtract sample background reading from sample reading.

10.3 Calculate the Citrate Synthase activity of the test sample $\Delta OD = A_2 - A_1$ during the reaction time ($\Delta T = T_2 - T_1$).

Sample CS activity = $B/(\Delta T \times V) \times D$ nmol/min/µL or mU/µL or U/mL

Where: **B** is the nanomoles of S-H group from Standard Curve.
ΔT is the reaction time (min.)

V is sample volume added into the reaction well (µL)

D is sample dilution factor

Sample citrate synthase activity can also be expressed as U/µg of protein.

Unit Definition: One unit of Citrate Synthase is the amount of enzyme that will generate 1.0 µmol CoA per min. at pH 7.2 at 25°C.

11. Typical Data

Typical data provided **for demonstration purposes only**.

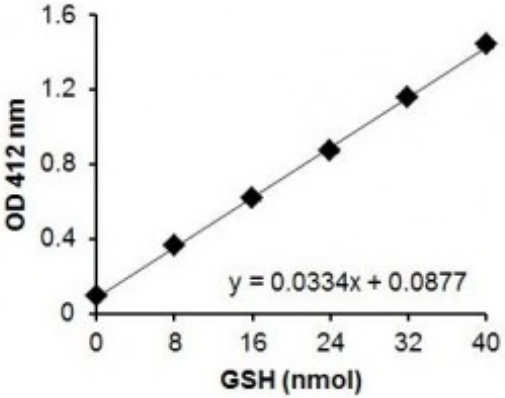

Figure 1. GSH Standard Curve.

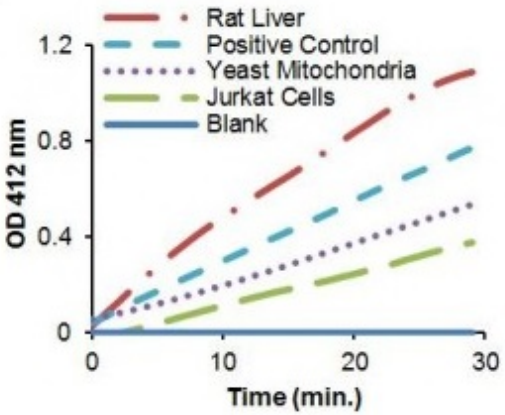

Figure 2. Citrate Synthase Activity in Jurkat cell lysate (10 µg), rat liver lysate (20 µg), purified yeast mitochondria (4 µg) and CS Positive Control (2 µl). Assays were performed following the kit protocol.

13. Notes

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