

Version 4a, Last updated 7 August 2025

# 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 DTNB 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  $\mu$ L of Reaction Mix to each well containing samples, Positive Control and Standards. Add 50  $\mu$ L 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](http://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
Assay Buffer 7	25 mL	-20°C
CS Substrate Mix	1 vial	-20°C
DTNB	1 vial	-20°C
GSH Standard (Reduced)	1 vial	-20°C
CS Positive Control	1 vial	-20°C

PLEASE NOTE: Assay Buffer 7 was previously labelled as Assay Buffer VII and CS Assay Buffer, and DTNB as CS Developer (Lyophilized).

## 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 Assay Buffer 7:

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

### 6.2 CS Substrate Mix:

Reconstitute with 220  $\mu\text{L}$  dH<sub>2</sub>O. Aliquot and store at -20°C. Keep on ice while in use. Use within two months.

### 6.3 DTNB:

Reconstitute with 1 mL Assay Buffer 7. 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  $\mu\text{L}$  dH<sub>2</sub>O 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  $\mu\text{L}$  Assay Buffer 7 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  $\mu\text{L}$  of 20 mM Standard to 90  $\mu\text{L}$  of Assay Buffer.
- 7.2** Add 0, 4, 8, 12, 16, 20  $\mu\text{L}$  of diluted GSH Standard into 96-well plate and adjust the volume to 50  $\mu\text{L}$  with Assay Buffer to generate 0, 8, 16, 24, 32 and 40 nmol GSH Standard/well.

Standard #	2mM GSH Standard ( $\mu\text{L}$ )	Assay Buffer ( $\mu\text{L}$ )	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 ( $1 \times 10^6$ ) on ice with 100  $\mu$ L ice cold Assay Buffer 7. 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  $\mu$ L sample into a 96-well plate. Adjust the volume to 50  $\mu$ L with Assay Buffer 7.
- 8.4 Alternatively, isolate mitochondria from fresh tissues or cells. Add 1-50  $\mu$ L of isolated mitochondrial sample into a 96-well plate and adjust the volume to 50  $\mu$ L with Assay Buffer 7.

### **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 10 times by adding 10  $\mu\text{L}$  of stock solution into 90  $\mu\text{L}$  of Assay Buffer 7. Add 20  $\mu\text{L}$  of diluted CS Positive Control into desired well(s) and adjust the volume to 50  $\mu\text{L}$  with Assay Buffer 7.
- 9.2 Prepare 50  $\mu\text{L}$  Reaction Mix for each well to be assayed as per the table and mix well. Add 50  $\mu\text{L}$  of Reaction Mix into Standard, Positive Control and sample wells. Mix well.

	Reaction Mix	Background Control Mix
Assay Buffer 7	43 $\mu\text{L}$	45 $\mu\text{L}$
DTNB	5 $\mu\text{L}$	5 $\mu\text{L}$
CS Substrate Mix	2 $\mu\text{L}$	-

**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 ( $\Delta T = T_2 - T_1$ ).

$$\text{Sample CS activity} = B / (\Delta T \times V) \times D \text{ nmol/min/}\mu\text{L or mU/}\mu\text{L or U/mL}$$

Where: **B** is the nanomoles of S-H group from Standard Curve.

**$\Delta T$**  is the reaction time (min.)

**V** is sample volume added into the reaction well ( $\mu\text{L}$ )

**D** is sample dilution factor

Sample citrate synthase activity can also be expressed as U/ $\mu\text{g}$  of protein.

Unit Definition: One unit of Citrate Synthase is the amount of enzyme that will generate 1.0  $\mu\text{mol}$  CoA per min. at pH 7.2 at 25°C.

## 11. Typical Data

Typical data provided for demonstration purposes only.

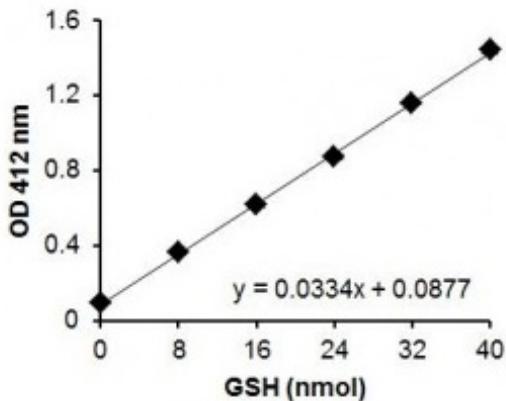


Figure 1. GSH Standard Curve.

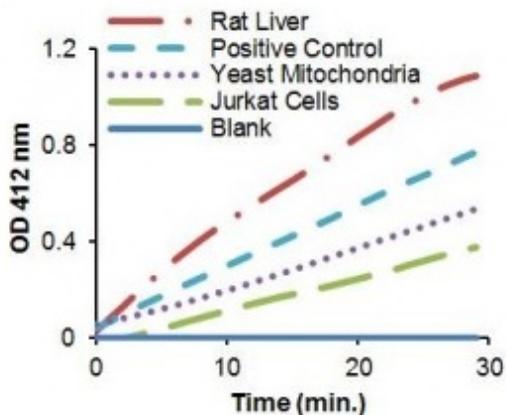


Figure 2. Citrate Synthase Activity in Jurkat cell lysate (10  $\mu$ g), rat liver lysate (20  $\mu$ g), purified yeast mitochondria (4  $\mu$ g) and CS Positive Control (2  $\mu$ l). Assays were performed following the kit protocol.



## 13. Notes





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