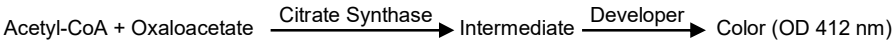
Citrate Synthase Activity Colorimetric Assay Kit (#BN00585)

(Catalog #BN00585; 100 assays; Store at -20°C)

I. Introduction:

Citrate Synthase (EC 2.3.3.1) is a key enzyme that is present in all living organisms. It catalyzes the conversion of acetyl-CoA and oxaloacetate into citrate and serves as a marker for intact mitochondria. A recent study showed that increased activity of mitochondrial Citrate Synthase is directly associated with metabolic and endocrine abnormalities such as obesity. In Assay Genie's Citrate Synthase Activity Assay Kit, 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.



II. Application:

- Measurement of citrate synthase activity in various tissues/cells
- Analysis of intact mitochondria

III. Sample Type:

- Animal tissues: liver, heart, kidney, etc.
- Cell culture: Adherent or suspension cells
- Purified mitochondria

IV. Kit Contents:

Components	BN00585	Cap Code	Part Number
CS Assay Buffer	25 ml	WM	BN00585-1
CS Substrate Mix (Lyophilized)	1 vial	Blue	BN00585-2
CS Developer (Lyophilized)	1 vial	Red	BN00585-3
GSH Standard (reduced) (Lyophilized)	1 vial	Yellow	BN00585-4
CS Positive Control (Lyophilized)	1 vial	Purple	BN00585-5

V. User Supplied Reagents and Equipment:

- 96-well clear plate with flat bottom
- Multi-well spectrophotometer (ELISA reader)

VI. Storage and Handling:

Store kit at –20°C, protected from light. Warm Assay Buffer to room temperature before use. Briefly centrifuge small vials prior to opening. Read entire protocol before performing the experiment.

VII. Reagent Preparation and Storage Conditions:

- **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.
- **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.
- **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.
- **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.

VIII. Citrate Synthase Activity Assay Protocol:

1. **Sample Preparation:** 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 min. Centrifuge at 10,000 X g for 5 min. Collect the supernatant. Add 1-50 µl sample into a 96-well plate. Adjust the volume to 50 µl with CS Assay Buffer. To isolate mitochondria from fresh tissues or cells, use Assay Genie's Mitochondria Isolation Kit (K288, K259). Add 1-50 µl of isolated mitochondrial sample into a 96-well plate & adjust the volume to 50 µl with CS Assay Buffer. 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) & adjust the volume to 50 µl with CS Assay Buffer.

   Note:

   a. For unknown samples, we suggest testing several doses to ensure the readings are within the Standard Curve range.

   b. For samples having high CoA level, prepare parallel sample well(s) as background control.

2. **Standard Curve Preparation:** Dilute GSH Standard to 2 mM by adding 10 µl of 20 mM Standard to 90 µl of Assay Buffer. Add 0, 4, 8, 12, 16, 20 µl of diluted GSH Standard into 96-well plate & adjust the volume to 50 µl with Assay Buffer to generate 0, 8, 16, 24, 32 & 40 nmol GSH Standard/well.

3. **Reaction Mix:** Mix enough reagents for the number of assays (Samples, background control, Positive Control & Standards) to be performed. For each well, prepare 50 µl mix containing:

Reaction Mix	* Background Control Mix

CS Assay Buffer	43 µl	45 µl
CS Developer	5 µl	5 µl
CS Substrate Mix	2 µl	---

Add 50 µl of Reaction Mix to each well containing samples, Positive Control and Standards.

* For samples having high CoA level, add 50 µl of Background Control Mix into sample background control well(s). Mix well.

4. **Measurement:** Measure absorbance (OD 412 nm) immediately in kinetic mode at 25ºC for 20-40 min.
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 (T_1 & T_2) in the linear range to calculate the Citrate Synthase Activity of the samples.

5. **Calculation:** Subtract 0 Standard reading from all readings. Plot the GSH Standard Curve. If sample background control reading is significant then subtract sample background reading from sample reading. 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 Citrate Synthase activity = B /(ΔT X V) X D = nmol/min/µl = 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 the sample volume added to reaction well (µl)
D is the 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 25ºC.

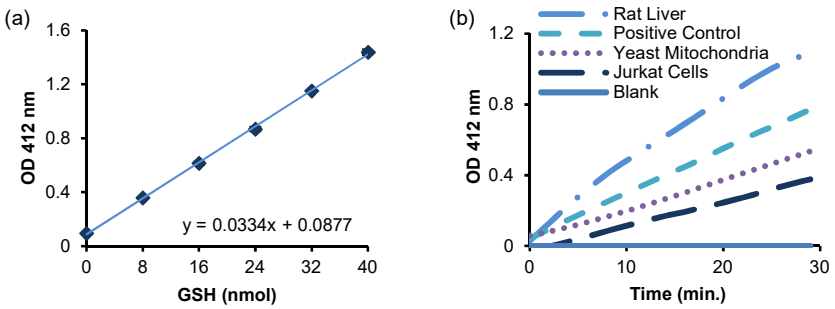


Figure: (a) GSH Standard Curve. (b) Citrate Synthase Activity in Jurkat cell lysate (10 µg), rat liver lysate (20 µg), purified yeast mitochondria (4 µg) and CS Positive Control (2 µl). Assays were performed following the kit protocol.

FOR RESEARCH USE ONLY! Not to be used on humans.