Abcam citrate synthase assay kit

Protocol
https://www.abcam.com/ps/products/239/ab239712/documents/ab239712_Citrate%20Synthase%20Assay%20Kit%20v1a%20(website).pdf

Protocol Summary

1. Prepare samples
2. Prepare GSH Standard curve (6 tubes)-measure at 412 nm once to get the curve.
3. Run plate with GSH standard, positive control and background control first. (once per day)
4. 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. (use extra sample volume)
5. Measure absorbance (OD 412 nm) immediately in kinetic mode at 25ºC for 20-40 mins.

Materials

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.**

- ☐ 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
- ☐ 96-well plate with flat bottom
- ☐ Multi-well spectrophotometer
- ☐ Pipette (p1000, p200, p100, p10)
- ☐ Repeater Combitips advanced (50uLx2.5mL) https://pipette.com/eppendorf-30089553.html
- ☐ Small volume tubes for aliquots (1.5mL)
- ☐ balance

Reagent Preparation

1. Before using the kit, spin tubes and bring down all components to the bottom of tubes.
2. Prepare only as much reagent as is needed on the day of the experiment.
    a. **6.1** CS Assay Buffer: Store at -20°C. Bring to room temperature (RT) before use.
    b. **6.2** CS Substrate Mix: Reconstitute with **220 µL dH2O (p1000)**. Aliquot and store at -20°C. Keep on ice while in use. Use within two months.

c. **6.3** CS Developer: Reconstitute with **1 mL CS Assay Buffer (p1000)**. Pipette up and down to dissolve completely. Aliquot and store at -20°C. Keep on ice while in use. Use within two months.
d. **6.4** GSH Standard (reduced): Reconstitute with **100 µL dH2O** (p200) to make **20 mM** GSH Standard solution. Aliquot and store at -20°C. Keep on ice while in use. Use within two months.
e. **6.5** CS Positive Control: Reconstitute with **100 µL CS Assay Buffer (p200)** to make the stock solution and mix thoroughly. Aliquot and store at 20°C. Keep on ice while in use. Use within two months.

Standard Preparation

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

1. Dilute GSH Standard to 2 mM by adding 10 µL of 20 mM Standard to 90 µL of Assay Buffer.
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 (50, 46, 42, 38, 34, 30 uL) to generate 0, 8, 16, 24, 32 and 40 nmol GSH Standard/well.**(p100x7, p10x5)**

Final concentration (50uL)	CS Assay Buffer	GSH (2mM) standard
0 nmol	50uL	0
8 nmol	46uL	4uL
16 nmol	42uL	8uL
24 nmol	38uL	12uL
32 nmol	34uL	16uL
40 nmol	30uL	20uL

Sample Preparation

1. Weigh tissue and record https://docs.google.com/spreadsheets/d/1_8vj1T2yd3Cd6FhpHUA-QPrzZVGvqAXz1wseTCH7Ihw/edit#gid=0
2. Homogenize tissue (10-20 mg) or cells (1 x 10^6) on ice with 350 µL ice cold CS Assay Buffer using **P1000**. Keep on ice for 10 mins. (use from BSA protocol)
3. Centrifuge at 10,000 x g for 5 mins. Collect the supernatant into new tubes and label them.
4. Collect BSA data (https://docs.google.com/document/d/1CZtU6QTkBIhtNHrl0dIxgEd1ubukJDFf64_rAckiHkY/edit)

5. Add 50 µL **(p200)** sample into a 96-well plate (Adjust the volume to 50 µL if < 50uL 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.***

Assay Procedure

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.(2uL PC + 48uL buffer) or (20uL PC + 30uL buffer)
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 using 2.5mL x 50uL combitips advanced or multichannel **p200**. Mix well. Use a plate spinner to eliminate bubbles, measured in triplicate. (for a 96 well plate, adding 96x50uL of reaction mix will take more than 20 minutes and takes it out of the reaction time, using single tipped pipettes either takes too long or produces too many bubbles).

	Reaction Mix (uL)	Background Control Mix (uL)
CS Assay Buffer	43	45
CS developer	5	5
CS substrate mix	2	-

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

3. Measure absorbance (OD 412 nm) immediately in kinetic mode at 25ºC 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.

Data Analysis

1. Subtract 0 Standard reading from all readings. Plot the GSH Standard Curve.
2. If sample background control reading is significant then subtract sample background reading from sample reading.
3. Calculate the Citrate Synthase activity of the test sample ΔOD = A2 – A1 during the reaction time (ΔT = T2 - T1).

Sample CS activity = B/(ΔT X V) X D nmol/min/µL or mU/µL or U/mL

Where:

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

Δ**T** 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 25ºC.

https://www.abcam.com/ps/products/239/ab239712/documents/ab239712_Citrate%20Synthase%20Assay%20Kit%20v1a%20(website).pdf

Example CS absorbance curve: length of exposure = 45 min, each repeat ~ 4min.
A=absorbance from spec machine output without correction