Product Information

Citrate Synthase Activity Colorimetric Assay Kit

I. Kit Contents:

Components	K2029-100 100 assays	Cap Color	Part Number
CS Assay Buffer	25 ml	WM	K2029-C-1
CS Substrate Mix (Lyophilized)	1 vial	Blue	K2029-C-2
CS Developer (Lyophilized)	1 vial	Red	K2029-C-4
GSH Standard (reduced) (Lyophilized)	1 vial	Yellow	K2029-C-5
CS Positive Control (Lyophilized)	1 vial	Purple	K2029-C-6

II. Introduction:

Citrate Synthase is a pace-making enzyme in the first step of the Citric Acid Cycle that catalyzes the conversion of acetyl-CoA and oxaloacetate into citrate and exists in all living organisms. Citrate synthase is localized in the mitochondrial matrix within eukaryotic cells and serves as a marker for intact mitochondria. The increase of mitochondrial citrate synthase activity is directly associated with endocrine and metabolic abnormalities such as obesity.

The Citrate Synthase Activity Colorimetric Assay Kit provides a simple and convenient way for the quantification of citrate synthase activity in a variety of biological samples. In the assay, citrate synthase reacts with CS substrate mix to generate an intermediate, which subsequently reacts with CS developer to generate the colored product, which can be easily detected by plate reader or spectrophotometer at $OD_{412\ nm}$. The rate of color produce is proportional to citrate synthase activity. The assay is simple, convenient and rapid, and can detect the enzyme activity less than 1 mU in a variety of biological samples.

III. Application:

Measurement of citrate synthase activity in various tissues/cells.

Analysis of intact mitochondria.

IV. Sample Type:

Animal tissues: liver, heart, kidney, etc.

Cell culture: Adherent or suspension cells.

Purified mitochondria.

V. User Supplied Reagents and Equipment:

96-well clear plate with flat bottom.

Multi-well spectrophotometer (ELISA reader).

VI. Reagent Preparation and Storage Conditions:

Substrate Mix: Dissolve with 220 µl Assay Buffer. Pipette up and down to completely dissolve. Store at –20℃. Use within two months.

Quench Remover: Dissolve in 220 µl dH_2O. Keep on ice while in use, store at -20℃.

Acetyl CoA Standard: Dissolve in 100 µl dH_2O to generate 10 mM (10 nmol/µl) Acetyl CoA.

Standard solution. Keep cold while in use. Store at -20℃.

VII. Reagent Preparation and Storage Conditions:

CS Substrate Mix: Reconstitute with 220 µl dH_2O. Aliquot and store at -20℃. 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℃. 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℃. 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℃. 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. 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 25℃ 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 (T1 & T2) 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 ΔOD = A2 - A1 during the reaction time (ΔT = T2 - T1).

Sample Citrate Synthase activity = B /(ΔT × 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 25℃.

For research use only! Not to be used in humans.

Our promise

If the product does not perform as described on this datasheet, we will offer a refund or replacement. For more details, please visit http://www.apexbt.com/ or contact our technical team.

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