QuantiChrom™ Citrate Synthase Assay Kit (DCST-100)

Rapid Colorimetric Determination of Citrate Synthase Activity

DESCRIPTION

CITRATE SYNTHASE (E.C. 2.3.3.1) is a pivotal enzyme that initiates the tricarboxylic acid (TCA) cycle. It catalyzes the condensation reaction between acetyl-CoA and oxaloacetic acid, yielding citric acid. Citrate synthase levels of cells and tissue are used as a guide in determining mitochondrial dysfunctions and cellular metabolism. High levels of citrate synthase activity indicate increased metabolic activity or cellular energy production. This can occur in situations where cells are actively metabolizing substrates for energy production, such as during periods of increased physical activity or in tissues with high energy demands, like muscle tissue. Conversely, low levels of citrate synthase activity may suggest reduced metabolic activity or energy production. This could be seen in situations where cellular metabolism is slowed down, such as during periods of rest or in conditions associated with mitochondrial dysfunction.

Simple, direct and automation-ready procedures for measuring citrate synthase activity are very desirable. BioAssay Systems' QuantiChrom™ Citrate Synthase Assay is based on an improved Ellman method, in which coenzyme A thiol produced by the action of citrate synthase forms a yellow color with 5,5'-dithiobis (2-nitrobenzoic acid). The intensity of the product color, measured at 412 nm, is proportionate to the enzyme activity in the sample.

APPLICATIONS

Direct assays of citrate synthase activity in cell lysates, tissues, and other biological samples.

KEY FEATURES

Sensitive and accurate. Linear detection range 0.50 to 120 U/L citrate synthase activity in 96-well plate assay.
Simple and convenient. The procedure involves adding a single working reagent and reading the optical density at 5 min and 10 min at room temperature.
High-throughput. Can be readily automated as a high-throughput 96-well plate assay for thousands of samples per day.

KIT CONTENTS (100 tests in 96-well plates)

Assay Buffer: 9 mL	**Reagent A: dried**
Reagent B: dried	**Reagent C: 600 µL**
Calibrator: 1.5 mL (equivalent to 352 U/L)	

Storage conditions. The kit is shipped at RT. Store all components at -20°C upon receiving. Shelf life: 6 months after receipt.

Precautions: Reagents are for research use only. Normal precautions for laboratory reagents should be exercised while using the reagents. Please refer to Material Safety Data Sheet for detailed information.

PROCEDURE

This assay is based on an enzyme-catalyzed kinetic reaction. Addition of Working Reagent should be quick and mixing should be brief but thorough. Use of a multi-channel pipettor is recommended.

Sample preparation. Tissue or cell lysates are prepared by brief sonication or homogenization in 0.1M phosphate buffer (pH 7.5), followed by centrifugation at 14,000 rpm for 5 min. Use supernatant for assay. Ideally, samples should be assayed fresh.

Reagent preparation: Dissolve Reagent A with 100 µL dH_2O, and dissolve Reagent B with 100 µL dH_2O. Prepare enough Working Reagent for each well by mixing 85 µL Assay Buffer, 1 µL Reagent A, 1µL Reagent B, and 5 µL Reagent C.

1. *Calibrator:* transfer 100 µL calibrator into a separate well of a clear bottom 96-well plate. Transfer 100 µL dH_2O into a separate well of a clear bottom 96-well plate.
2. *Samples:* add 10 µL of each sample to separate wells.
3. *Reaction*: transfer 90 µL freshly prepared Working Reagent to the sample wells and tap plate briefly to mix.

   Read OD_{412nm} at 5 min and at 10 min.

Calculation: citrate synthase activity is calculated as follows:

$$\text{Citrate Synthase Activity} = \frac{OD_{10} - OD_5}{OD_{CAL} - OD_{H2O}} \times 352 \times n \text{ (U/L)}$$

Where OD_{10} and OD_5 are the OD_{412nm} values of the sample at 10 min and 5 min, respectively. OD_{CAL} and OD_{H2O} are the OD_{412nm} values of the Calibrator and dH_2O at 10 min. *n* is the dilution factor. *t* is the reaction time (10min - 5 min = 5 min is the recommended time). The number "352" is the equivalent activity of the Calibrator under the assay conditions.

Note: if the calculated citrate synthase activity is higher than 120 U/L, dilute sample in Assay Buffer and repeat the assay. Multiply the results by the dilution factor.

Unit definition: one unit of enzyme catalyzes the production of 1 µmole of coenzyme A per minute under the assay conditions (pH 8.0 and room temperature).

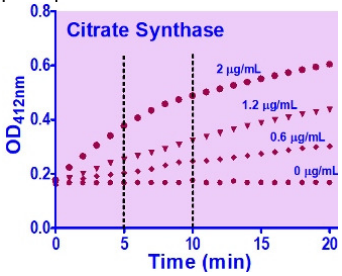
MATERIALS REQUIRED, BUT NOT PROVIDED

Pipeting (multi-channel) devices. Clear-bottom 96-well plates (e.g. Corning Costar) and plate reader capable of reading at 412nm.

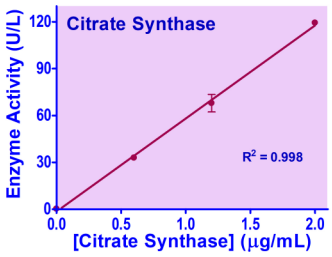
EXAMPLES

Duplicate assays for lysates of Hep G2 cells, PANC-1 cells, HeLa cells, Jurkat cells, mouse brain and stomach gave citrate synthase content of 28.4 ± 5.6, 149.9 ± 18.3, 31.3 ± 4.4, 43.1 ± 5.6, 2.4 ± 0.1 and 6.2 ± 2.1 U/*g* total protein.

Citrate synthase from E. coli was assayed in triplicate using the 96-well plate protocol.



Raw Kinetics of Citrate Synthase



Standard Curve

LITERATURE

1. Sumi, K et al (2022). Citrate Synthase Insufficiency Leads to Specific Metabolic Adaptations in the Heart and Skeletal Muscles Upon Low-Carbohydrate Diet Feeding in Mice. Frontiers in Nutrition, 9:925908.
2. Chhimpa, N et al (2023). The Novel Role of Mitochondrial Citrate Synthase and Citrate in the Pathophysiology of Alzheimer's Disease. Journal of Alzheimer's Disease. 94(s1):S453–S472.
3. Alhindi, Y et al (2019). Low Citrate Synthase Activity Is Associated with Glucose Intolerance and Lipotoxicity. Journal of Nutrition and Metabolism. 2019:8594825.