ABSbio™ Citrate Synthase Activity Detection Kit

(Cat# K298-100; 100 assays; store kit at -20℃)

Introduction

Citrate Synthase (CS) 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. CS is localized in the mitochondrial matrix within eukaryotic cells and serves as a marker for intact mitochondria. A recent study showed that increased activity of mitochondrial CS is directly associated with metabolic and endocrine abnormalities.

The ABSbio™ Citrate Synthase (CS) Activity Detection Kit provides a simple, sensitive, one-step high-throughput adaptable colorimetric assay to detect CS activity in the various biological samples. In this assay, Citrate Synthase reacts with CS substrate to form an intermediate, which subsequently reacts with probe to generate the colored product. The intensity of color, measured at 412 nm, is directly proportional to the CS activity which is present in the sample. The kit is supplied with sufficient reagents for 100 tests in 96-well plate assay. It could easily be modified for use in 384-well assay and high-throughput assay.

Kit Components (100 tests)

Assay Buffer: 15 mL Substrate A: 120 µL Substrate B: 120 µL Probe: 120 µL
CS positive: 50 µL

Storage and Handling: Store kit at -20℃. Shelf Life: 6 months after receipt. Warm up Assay Buffer & Probe to room temperature before use.

Protocol

Keep CS positive on ice.

1. Sample preparation

Biological samples such as isolated mitochondria, serum, plasma should be directly assay by a series of dilutions of the sample to ensure the readings are within the standard curve range. Homogenize Cell (2 x 10^6) or tissue (20 mg) sample in 200 µL ice-cold Assay Buffer. Centrifuge to collect the supernatant. It is recommended with all sample types to assay immediately or aliquot and store the sample at -80°C.

Transfer 20 µL sample (optional: 10x diluted CS positive control with assay buffer) into the clear 96-well plate in duplicate. The blank control containing 20 µL buffer only.

2. Reaction

Prepare enough working reagent by mixing 80 µL assay buffer, 1 µL substrate A, 1 µL substrate B and 1 µL probe for each reaction. If sample contain CoA, please prepare sample blank working reagent by mixing 81 µL assay buffer, 1 µL substrate B and 1 µL probe for each sample. The assay is based on an enzyme-catalyzed kinetic reaction, using a multi-channel pipettor is recommended to transfer 80 µL prepared working reagent into each reaction well quickly. Tap plate to mix well.

3. Measurement

Immediately read the optical density in kinetic mode (30 second intervals)) at 412 nm. Since the activity of the enzyme increases with time, it is recommended to read the plate at several time points (e.g. t1 and t2 at 5 and 20 minutes). Then choose the data in the reaction linear range.

5. Calculation

Average the duplicate OD412 nm reading for sample. Subtract the average OD of the blank from the average OD of the sample, then plot data as ΔOD_{sample} (y-axis) *versus* time (in minutes) (x-axis).

Note: if has sample blank, the sample blank reading must be subtracted from sample readings.

Calculate the CS activity of sample as equation

$$[\text{CS activity}] = \frac{(OD_{t2\ sample} - OD_{t1\ sample}) / (t2-t1)}{\varepsilon \times l} \times \frac{\text{reaction volume (mL)}}{\text{sample volume (mL)}} \times n$$

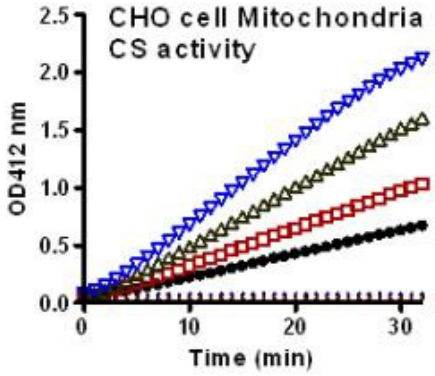
$$= \frac{1.23 \times (OD_{t2\ sample} - OD_{t1\ sample})}{t2-t1} \times n \ (\mu M/min/mL)$$

$$\text{Or by sample weight} = \frac{0.25 \times (OD_{t2\ sample} - OD_{t1\ sample})}{(t2-t1) \times mg\ sample} (\text{Unit/mg})$$

t is the reaction time (t_2-t_1 minutes), *n* is the sample dilution factor. **ε** is extinction coefficient of TNB 14.1 /mM/cm. ***l*** is path length 0.3 cm. Reaction volume is 0.1 mL, sample volume is 0.02 mL.

Unit Definition: One unit is the amount of CS that will generate 1.0 µM of CoA per min at 25℃ pH 7.4.

Typical CS activity in mitochondria



CHO cell Mitochondrial sample in 96 wells-plate assay

Sensitivity and Limit of Detection

The Limit of Detection was determined as 0.05 µM, and linear detection range up to 4 µM CoA in 96-well plate colorimetric assay. Sensitivity was determined as 0.1 U/L of CS. Samples with values above 4 µM should be dilute with assay buffer, re-assayed, and multiply results by dilution factor.

Interferences

Culture media contain phenol red in DMEM (15mg/L) and RPMI 1640 (5mg/L) were tested in the assay for interference in assay buffer. No significant change in the measured CS level was observed. Avoid samples containing DTT or β-mercaptoethanol since the probe is not stable in the presence of thiols (above 10 µM).

Related Products:

Mitochondria Activity Assay Kit (K658-100)

Mitochondria Isolation Kit (K608-100)