Rev. 2

ScienCell™
Research Laboratories

Citrate Synthase Assay (CS)

Catalog #8318
100 tests in 96-well plate

Product Description

Citrate synthase is the initial enzyme of the tricarboxylic acid (TCA) cycle. This enzyme catalyzes the reaction between acetyl coenzyme A (acetyl CoA) and oxaloacetic acid to form citric acid and CoA with a thiol group (CoA-SH). This colorimetric assay is based on the reaction between 5, 5'-Dithiobis 2-nitrobenzoic acid (DTNB) and CoA-SH to form TNB, which exhibits maximum absorbance at 412 nm. The intensity of the absorbance is proportional to the citrate synthase activity. This enzyme is an exclusive marker of the mitochondrial matrix[1].

Kit Components

Cat. No.	# of vials	Reagent	Quantity	Storage
8318a	1	Assay Buffer (5X)	5 mL	4°C
8318b	1	Acetyl CoA	1 mL	-20°C
8318c	1	DTNB (2X)	0.3 mL	-20°C
8318d	1	10% Triton X-100	0.2 mL	4°C
8318e	1	Oxaloacetate	40 mg	-20°C

Reagents and Equipment Supplied by User

1. 96-well plate reader
2. Ultrapure water (ScienCell™ Cat. #0600)
3. Mitochondria isolation kit (ScienCell™ Cat. #8268)

Quality Control

Mitochondria citrate synthase activity was measured on mitochondria from primary cells in serial dilution. The citrate synthase activity (OD_{412nm}) is proportional to the amount of mitochondria in reaction.

Product Use

This kit is used for the fast and simple measurement of citrate synthase activity as well as the detection of intact mitochondrial inner membrane. It is for research purposes only and not for use in animals, humans, or diagnostic procedures.

Shipping

Dry ice.

Sample and Buffer Preparation

1. Mitochondria isolation: Isolate mitochondria from cultured cells or tissue by using mitochondria isolation kit (ScienCell™ Catalog #8268).
2. Assay buffer solution (1X): Dilute assay buffer (Cat. #8318a) in ultra-pure water (1:4).
3. DTNB solution (1X): Dilute DTNB (Cat. #8318c) in ultra-pure water (1:1).
4. Oxaloacetate solution: Dissolve 1.32mg (Cat. #8318e) per mL 1x assay buffer, mix until homogenous. This solution can be stored at -20°C for up to one week.

Procedure for Citrate Synthase Activity Assay (96-well plate)

1. Set the spectrophotometer at 412 nm on a kinetic program:
   Duration: 10 minutes
   Interval: 30 seconds
2. Warm the assay solutions to room temperature before starting the reaction. Mix until homogenous.
3. Prepare sample reactions for each well according to the reaction scheme (see Table 1).

Table 1. Reaction Mixes.

Assay Buffer 1X	185-x µL
Acetyl CoA	10 µL
DTNB solution 1X	3 µL
Triton X-100	2 µL
Mitochondrial protein (1~10 µg)	x µL

4. Add 10 µL oxaloacetate solution to each well and mix.
5. **Immediately** read and record increase in OD every minute for 10 minutes.
6. Calculate ΔA/min by using of the maximum linear rate. ΔA= change in OD reading.
7. Calculate citrate synthase activity of the sample (see calculations).

Calculations

Calculate the citrate synthase activity using the following equation:

$$\text{Unit/mg mitochondria} = \frac{\Delta A/\text{min}}{\varepsilon \times L(\text{cm}) \times \text{mg mitochondria}}$$

ΔA/min = (change in OD reading)/time
$\varepsilon = 13.6\ mM^{-1}\ cm^{-1}$ and ε is extinction coefficient of TNB at 412 nm
L (cm) = path length for absorbance
For 96-well plate, path length = 0.625 cm

Unit definition: One unit would make 1.0 µmole DTNB become TNB per minute at pH 7.2 at 25 ºC

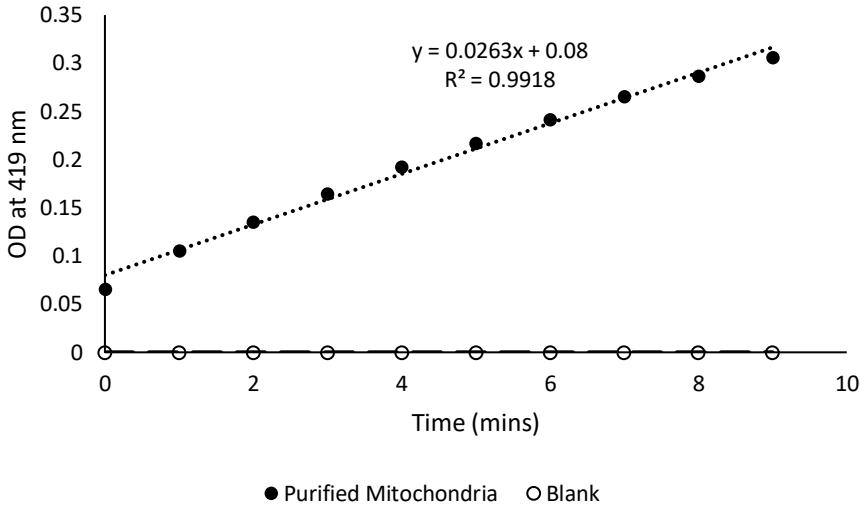

Figure 1. Citrate Synthase Activity of purified mitochondria (8µg) from Smooth Muscle Cells.

Procedure for Measuring Mitochondrial inner Membrane Integrity

Citrate synthase locates in the matrix of the mitochondria. The integrity of the mitochondrial inner membrane is assessed by measuring citrate synthase activity in the presence and absence of the detergent, triton X-100. The ratio between activity without and with triton X-100 presence is a measurement of the integrity of the mitochondrial inner membrane. Freeze/thaw processes may potentially cause rupture of the membrane of mitochondria. Therefore, freshly prepared tissues are recommended, though frozen tissues could still be used for measuring total activity of citrate synthase.
Note: To assess intact mitochondria, replace the 2 µl Triton X with 2 µl assay buffer in the reaction mix indicated in Table 1.

Percentage of mitochondria with intact inner membrane:

$$\% = \frac{\Delta A/\text{minute(w/ detergent)} - \Delta A/\text{minute (w/o detergent)}}{\Delta A/\text{minute (w/ detergent)}}$$

ΔA= change in OD reading
w/= With
w/o= Without

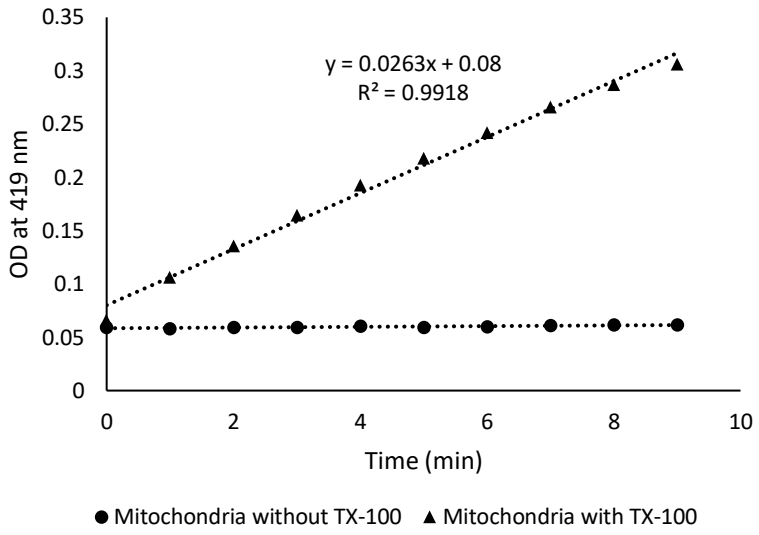

Figure 2. Using Citrate Synthase to calculate integrity of inner membrane of mitochondria.

Example: Calculation of the percentage of mitochondria with intact inner membrane:

$$\Delta A/\text{minute}(\text{w/detergent}) = 0.3095 - 0.066 = 0.2435$$

$$\Delta A/\text{minute}(\text{w/o detergent}) = 0.062 - 0.0595 = 0.0025$$

$$\% = \frac{0.2435 - 0.0025}{0.2435} = 0.989733 * 100 = 98.97\%$$

References:

1. Morgunov, I., & Srere, P. A. (1998). Interaction between citrate synthase and malate dehydrogenase: substrate channeling of oxaloacetate. *Journal of Biological Chemistry*, *273*(45), 29540-29544.
2. Trounce, I. A., Kim, Y. L., Jun, A. S., & Wallace, D. C. (1996). Assessment of mitochondrial oxidative phosphorylation in patient muscle biopsies, lymphoblasts, and transmitochondrial cell lines. In *Methods in enzymology* (Vol. 264, pp. 484-509). Academic press.