ScienCell™
Research Laboratories

Citrate Synthase Assay (CS)

Cat. No. 8318
50 tests in cuvette or 250 tests in 96-well plate

Introduction

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)	12.5 mL	4°C
8318b	1	Acetyl CoA	2.5 mL	-20°C
8318c	1	DTNB (2X)	0.75 mL	-20°C
8318d	1	Triton X-100	0.5 mL	4°C
8318e	1	Oxaloacetate	0.1 g	-20°C

Product Use

This kit is used for the fast and simple measurement of citrate synthase activity[2] 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.

Quality Control

Mitochondria citrate synthase activity was measured on rodent liver tissue and human dermal fibroblasts (ScienCell™ Cat.No.2300) derived mitochondria in serial dilution. The citrate synthase activity (OD_{412nm}) is proportional to the amount of mitochondria in reaction.

Reagents and Equipment Supplied by User

1. Spectrophotometer
2. 1mL cuvettes
3. 96-well plate reader
4. Ultrapure water (ScienCell™ Cat. No. 0600)
5. Mitochondria isolation kit (ScienCell™ Cat. No. 8268)

Shipping

Shipped on dry ice.

Sample and Buffer Preparation

1. Mitochondria isolation: isolate mitochondria from cultured cells or tissue by using mitochondria isolation kit (ScienCell™ Cat.No.8268).
2. Assay buffer solution (1X): dilute assay buffer (8318a) in ultra-pure water (1:4).
3. DTNB solution (1X): dilute DTNB (8318c) in ultra-pure water (1:1).
4. Oxaloacetate solution: dissolve 1.32mg (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 (1mL cuvette)

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

Assay Buffer (1X)	875-X µL
Acetyl CoA	50 µL
DTNB solution (1X)	15 µL
Triton X-100	10 µL
Mitochondrial protein (5~10 µg)	X µL

4. Mix solution in cuvette.
5. Blank spectrophotometer with reaction mixture.
6. Add 50 µL oxaloacetate solution and mix (cover with parafilm and invert 3-4 times).
7. **Immediately** read and record decrease in OD for 2 minutes.
8. Calculate ΔA/min by using of the maximum linear rate. ΔA= change in OD reading.
9. Calculate citrate synthase activity of the sample (see calculations).

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

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

Assay Buffer (1X)	175-X µL
Acetyl CoA	10 µL
DTNB solution (1X)	3 µL
Triton X-100	2 µL
Mitochondrial protein (1~2 µg)	X µL

4. Add 10 µL oxaloacetate solution and mix.
5. **Immediately** read and record decrease in OD for 2 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 1ml cuvette, path length = 1 cm
- 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

Example: Calculate the citrate synthase activity of isolated mitochondria from human smooth muscle cells.

2 µg mitochondria protein was used for citrate synthase activity assay in 96-well plate
The following are the absorbance readings:

Time point (second)	Absorbance at 412 nm
	0.49668
10	0.49848
20	0.50449
30	0.51020
40	0.51505
50	0.52149
60	0.52479
70	0.52879
80	0.53157
90	0.53313
100	0.53695
110	0.53852
120	0.54187

Calculate the linear rate during different time frame:

Time Frame	Linear Rate (ΔA/min)
T_{0s} to T_{30s}	0.027
T_{30s} to T_{60s}	0.029
T_{60s} to T_{90s}	0.017
T_{90s} to T_{120s}	0.017

The linear rate for human smooth muscle cells would be

$$\Delta A/min = \frac{0.027+0.029+0.017+0.017}{4} = 0.0225$$

Human smooth muscle cells citrate synthase activity would be

$$\text{Unit/mg mitochondria} = \frac{0.0225}{13.6 \times 0.625 \times 0.002} = 1.323 \text{ Unit/mg mitochondria}$$

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.

% mitochondria with intact mitochondria 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

References:

1. Morgunov I and Srere PA. Interacttion between citrate synthase and malate dehydrogenase substrate channeling of oxaloacetate. 1998. J. Biol. Chem 273: 29540-29544
2. Trounce IA, Kim YL, Jun AS, Wallace DC. Assessment of mitochondrial oxidative phosphorylation in patient muscle. biopsies, lymphoblasts, and transmitochondrial cell lines. 1996. Methods Enzymol 264: 484–509