A convenient, easy-to-follow shortened protocol is provided with this assay.
For a detailed protocol go to www.caymanchem.com/pdfs/701040.pdf

MitoCheck® Citrate Synthase Activity Assay Kit Short Protocol

Item No. 701040

REAGENT PREPARATION

1. **Citrate Synthase Oxaloacetate Reagent - (Item No. 701046)** - This reagent is supplied as a lyophilized powder. Suspend in 120 µl of UltraPure water and mix well prior to use.

2. **Citrate Synthase Acetyl Co-A Reagent - (Item No. 701048)** - This reagent is supplied as a lyophilized powder. Suspend in 120 µl of UltraPure water and mix well prior to use.

3. **Citrate Synthase Developer Reagent - (Item No. 701047)** - This reagent is supplied as a lyophilized powder. Suspend in 120 µl of UltraPure water and mix well prior to use.

BUFFER PREPARATION

Label two polystyrene tubes as A and B. Then add the following reagents. *Because samples can settle over time, make the sure contents of each tube are well mixed. Store tubes on ice until ready to use. Volumes indicated below are for 1 ml of buffer (20 wells), customer may scale volumes as needed.*

Tube A (1 mL)	Tube B (0.5 mL)
20 µl of Acetyl-CoA Reagent	20 µl of Oxaloacetate Buffer
20 µl of Developer Reagent	480 µl of Assay Buffer
960 µl of Assay Buffer	

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PERFORMING THE ASSAY

Sample Prep - A starting dilution of 1:200 (5 µl of sample into 995 µl of Assay Buffer) of the neat sample is recommended. It is the customers responsibility to determine an appropriate concentration to establish a linear rate.
Positive Control - Dilute by adding 5 µl to 5 ml (1:1,000) of Assay Buffer. Mix gently by inversion. Then add 10 µl of the 1:1,000 dilution to 990 µl of Assay Buffer. Mix gently by inversion. Store on ice until use in the assay.
For each assay condition:

1. Add 50 µl of the contents of tube A to each well.
2. Add 30 µl of diluted sample or positive control to each well. Quickly centrifuge plate if bubbles are present.
3. Add 20 µl of the contents of tube B to each well to start the reaction.

Immediately place plate on plate reader and measure absorbance at 412 nm (30 second intervals for 20 min @ 25°C).

CALCULATIONS

1. Plot data as absorbance (y-axis) *versus* time (in minutes) (x-axis).
2. To calculate the reaction rate, calculate the slope for the linear portion of the curve.
3. To quantify the reaction rate, use the equation below:

$$\left[\frac{\text{Reaction Rate}}{5.712\ \text{mM}^{-1**}} \times \frac{0.1\ \text{ml}}{0.03\ \text{ml}}\right] \times \text{Sample dilution} = \mu\text{mols/min/ml}$$

**5.712 is the extinction coefficient of DTNB (13.60 mM^{-1} cm^{-1}) after compensating for path length of the well. This equation will only function when used with the provided ½ volume 96-well plate (Item No. 700020). One unit of citrate synthase will turn over 1 µmol of developer per minute at 25°C, pH 7.4. To determine nmols/min/mg protein, divide nmols/min/ml by sample concentration (mg/ml).