MitoCheck® Citrate Synthase Activity Assay Kit

Item No. 701040

www.caymanchem.com
Customer Service 800.364.9897
Technical Support 888.526.5351
1180 E. Ellsworth Rd · Ann Arbor, MI · USA

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GENERAL INFORMATION

Materials Supplied

The kit will arrive in two different packages. The Citrate Synthase Activity Positive Control should be stored at 4°C. For best results, remove components and store as stated below.

Item Number	Item	Quantity/Size	Storage
701041	Citrate Synthase Activity Assay Buffer	2 vials/15 ml	4°C
701046	Citrate Synthase Oxaloacetate Reagent	1 vial/300 µg	-20°C
701048	Citrate Synthase Acetyl Co-A Reagent	1 vial/3 mg	-20°C
701047	Citrate Synthase Developer Reagent	1 vial/3 mg	-20°C
701045	Citrate Synthase Activity Positive Control	1 vial/30 µl	4°C*
700020	Half Volume 96-Well Clear Plate	1 plate	RT

*Avoid freezing

If any of the items listed above are damaged or missing, please contact our Customer Service department at (800) 364-9897 or (734) 971-3335. We cannot accept any returns without prior authorization.

WARNING: THIS PRODUCT IS FOR RESEARCH ONLY - NOT FOR HUMAN OR VETERINARY DIAGNOSTIC OR THERAPEUTIC USE.

Safety Data

This material should be considered hazardous until further information becomes available. Do not ingest, inhale, get in eyes, on skin, or on clothing. Wash thoroughly after handling. Before use, the user must review the complete Safety Data Sheet, which has been sent *via* email to your institution.

Precautions

Please read these instructions carefully before beginning this assay.

NOTE: It is recommended that gloves be worn at all time when working with isolated mitochondria and mitochondrial inhibitors.

If You Have Problems

Technical Service Contact Information

Phone: 888-526-5351 (USA and Canada only) or 734-975-3888

Fax: 734-971-3641

Email: techserv@caymanchem.com

Hours: M-F 8:00 AM to 5:30 PM EST

In order for our staff to assist you quickly and efficiently, please be ready to supply the lot number of the kit (found on the outside of the box).

Storage and Stability

This kit will perform as specified if stored as directed in the **Materials Supplied** section on page 3 and used before the expiration date indicated on the outside of the box.

Materials Needed But Not Supplied

1. A plate reader with the ability to measure absorbance at 412 nm with 30 second kinetic intervals
2. Adjustable and multichannel pipettes
3. A source of pure water; glass distilled water or HPLC-grade water is acceptable

INTRODUCTION

Background

The condensation of the dicarboxylate oxaloacetate and acetyl CoA to the tricarboxylate citrate is catalyzed by citrate synthase. It is within this reaction that carbon molecules (as acetyl CoA) obtained from pyruvate oxidation are fed into the tricarboxylic acid (TCA or citric acid) cycle. As a mitochondrial enzyme, citrate synthase is commonly used as a normalization factor for mitochondrial protein, but can also be used as a biomarker for mitochondrial content in a tissue homogenate.[1,2]

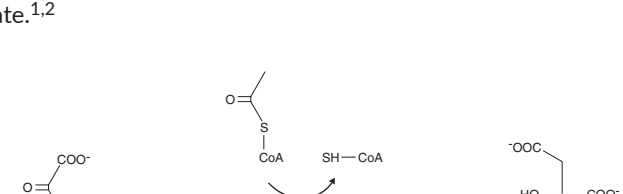


Figure 1. Scheme

About This Assay

Cayman's MitoCheck® Citrate Synthase Assay Kit allows for the simple and convenient determination of citrate synthase activity from isolated mitochondria, tissue, or cell homogenates. This assay measures the production of SH-CoA by monitoring the absorbance of Citrate Synthase Developing Reagent at 412 nm in a convenient 96-well format.

PRE-ASSAY PREPARATION

Reagent Preparation

All assay reagents, unless listed below, are ready to use as supplied. Any reconstituted reagents are stable for six hours on ice and must be used within this time period.

1. **Citrate Synthase Oxaloacetate Reagent - (Item No. 701046)**

   This reagent is supplied as a lyophilized powder. Reconstitute 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. Reconstitute 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. Reconstitute in 120 µl of UltraPure water and mix well prior to use.

ASSAY PROTOCOL

Buffer Preparation

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

Tube A	Tube B
20 µl of Acetyl-CoA Reagent	20 µl of Oxaloacetate Reagent
20 µl of Developer Reagent	480 µl of Assay Buffer
960 µl of Assay Buffer	

Table 1. Buffer preparation

Pipetting Hints

- Use different tips to pipette each reagent.
- Avoid introducing bubbles into the well.
- Do not expose the pipette tip to the reagent(s) already in the well.

Performing the Assay

Sample Preparation

For unknown concentrations of isolated mitochondrial protein or tissue homogenate it is recommended that a starting dilution of 1:200 (5 µl of sample into 995 µl of Assay Buffer) of the neat sample be used. It is however the customers responsibility to determine an appropriate concentration to establish a linear enzymatic rate in the assay.

Preparation of the Positive Control

Dilute the Citrate Synthase Assay Positive Control by adding 10 µl to 1.75 ml of Assay Buffer (primary dilution). Mix thoroughly by gentle inversion. Next, add 10 µl of the primary dilution to 990 µl of Assay Buffer (secondary dilution). Mix gently by inversion. This secondary dilution can now be used as described below (*e.g.*, 30 µl of Positive Control can be added to wells designated for the Positive Control). Store on ice until use.

For each assay condition

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

Immediately measure the absorbance at 412 nm in a plate reader every 30 seconds for 20 minutes at 25°C.

ANALYSIS

Calculations

1. Plot the absorbance (y-axis) *versus* time (in minutes) (x-axis).
2. To calculate the reaction rate, determine 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 containing 100 µl (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 specific activity (µmols/min/mg protein) divide nmols/min/ml by sample concentration (mg/ml).

Performance Characteristics

Sample Data

The results shown below are an example of data obtained with this kit. Your results will not be identical to these. Do not use these data to directly compare your samples as your results may vary substantially.

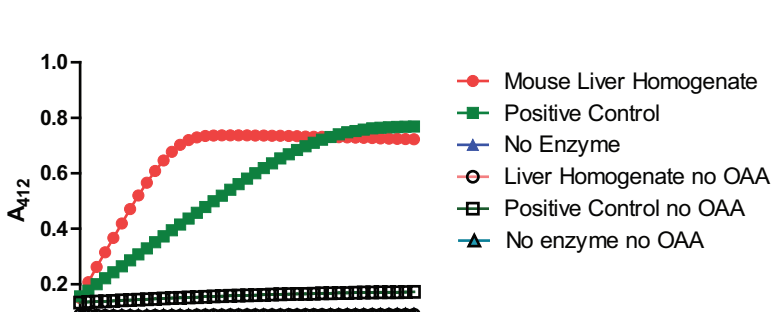


Figure 2. Sample data obtained using the MitoCheck® Citrate Synthase Assay Kit.

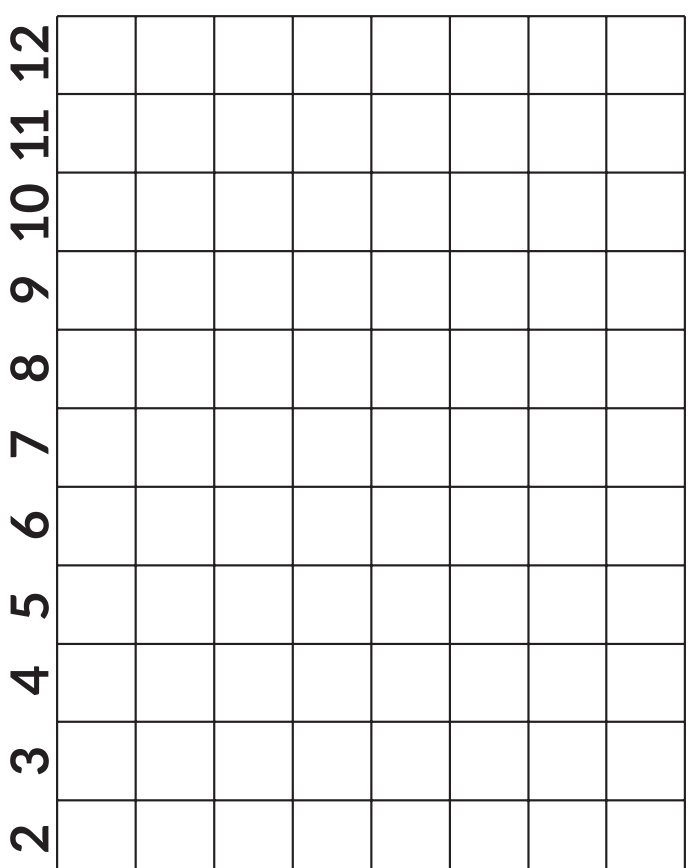
RESOURCES

Troubleshooting

Problem	Possible Causes	Recommended Solutions
Erratic values; dispersion of duplicates/triplicates	A. Poor pipetting/technique B. Bubble in the well(s)	A. Be careful not to splash the contents of the wells B. Centrifuge to remove the bubbles
No activity was detected in sample wells	Improper handling of samples; avoid multiple freeze thaw cycles of samples; samples should be kept on ice	Ensure activity of Positive Controls to confirm the kit is functioning normally

References

1. López-Lluch, G., Hunt, N., Jones, B., *et al.* Calorie restriction induces mitochondrial biogenesis and bioenergetic efficiency. *Proc. Natl. Acad. Sci. USA* **103(6)**, 1768-1773 (2006).
2. Wiegand, G. and Remington, S.J. Citrate synthase: Structure, control, and mechanism. *Annu. Rev. Biophys. Biophys. Chem.* **15**, 97-117 (1986).



NOTES

Warranty and Limitation of Remedy

Buyer agrees to purchase the material subject to Cayman's Terms and Conditions. Complete Terms and Conditions including Warranty and Limitation of Liability information can be found on our website.