

**MitoCheck™ Citrate Synthase
Activity Assay Kit**

Item No. 701040



Customer Service 800.364.9897 * **Technical Support** 888.526.5351
www.caymanchem.com

TABLE OF CONTENTS

GENERAL INFORMATION	3	Materials Supplied
	4	Precautions
	4	If You Have Problems
	4	Storage and Stability
	4	Materials Needed but Not Supplied
INTRODUCTION	5	Background
	5	About This Assay
PRE-ASSAY PREPARATION	6	Reagent Preparation
ASSAY PROTOCOL	7	Buffer Preparation
	8	Performing the Assay
ANALYSIS	9	Calculations
	10	Performance Characteristics
RESOURCES	11	Troubleshooting
	12	References
	13	Related Products
	14	Warranty and Limitation of Remedy
	15	Plate Template
	16	Notes

GENERAL INFORMATION


Materials Supplied

The kit will arrive as 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/1 mg	-20°C
701047	Citrate Synthase Developer Reagent	1 vial/3 mg	-20°C
701045	Citrate Synthase Activity Positive Control	1 vial/10 µl	4°C*
700020	Half Volume 96-Well Clear Plate	1 plate	Room temperature

*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 laboratory research use only; not for administration to humans. Not for human or veterinary diagnostic or therapeutic use.

Precautions

Please read these instructions carefully before beginning this assay.

For research use only. Not for human or diagnostic use.

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 capable of measuring absorbance at 412 nm at 30 second 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}

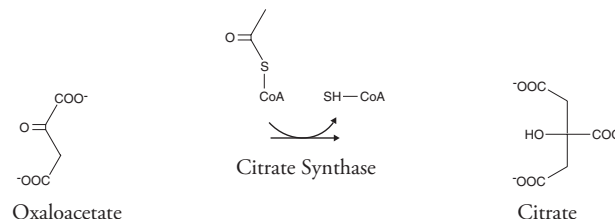


Figure 1. Scheme

About This Assay

Cayman's MitoCheckTM Citrate Synthase Assay Kit allows for the simple and convenient determination of citrate synthase activity from isolated mitochondria 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.

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.

ASSAY PROTOCOL

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 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 5 µl to 5 ml of Assay Buffer (*i.e.*, 1:1,000-fold dilution). Mix gently by inversion. Following this, add 10 µl of the 1:1,000 Positive Control dilution to 990 µl of Assay Buffer. Mix gently by inversion. This 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 contents of 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 minutes at 25°C).

ANALYSIS

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:

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

******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 specific activity (nmols/min/mg protein) divide nmols/min/ml by sample concentration (mg/ml).

Performance Characteristics

Sample Data

The data 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.

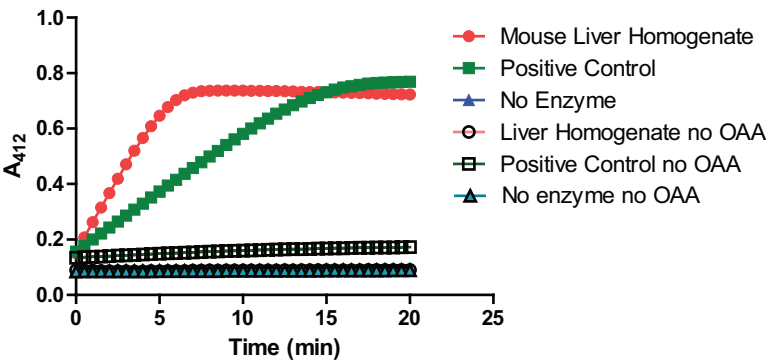


Figure 2. Sample data obtained using the MitoCheck™ Citrate Synthase Assay Kit.

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 ensure that 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).

Related Products

Aconitase Assay Kit - Item No. 705502
Aconitase Fluorometric Assay Kit - Item No. 700600
JC-1 Mitochondrial Membrane Potential Assay Kit - Item No. 10009172
MitoCheck™ Complex I Activity Assay Kit - Item No. 700930
MitoCheck™ Complex II Activity Assay Kit - Item No. 700940
MitoCheck™ Complex II/III Activity Assay Kit - Item No. 700950
MitoCheck™ Complex IV Activity Assay Kit - Item No. 700990
MitoCheck™ Complex V Activity Assay Kit - Item No. 701000
MitoCheck™ Mitochondrial (Tissue) Isolation Kit - Item No. 701010
Oxygen Consumption Rate Assay Kit (MitoXpress®-Xtra HS Method) - Item No. 600800

Warranty and Limitation of Remedy

Cayman Chemical Company makes **no warranty or guarantee** of any kind, whether written or oral, expressed or implied, including without limitation, any warranty of fitness for a particular purpose, suitability and merchantability, which extends beyond the description of the chemicals hereof. Cayman **warrants only** to the original customer that the material will meet our specifications at the time of delivery. Cayman will carry out its delivery obligations with due care and skill. Thus, in no event will Cayman have **any obligation or liability**, whether in tort (including negligence) or in contract, for any direct, indirect, incidental or consequential damages, even if Cayman is informed about their possible existence. This limitation of liability does not apply in the case of intentional acts or negligence of Cayman, its directors or its employees.

Buyer’s **exclusive remedy** and Cayman’s sole liability hereunder shall be limited to a refund of the purchase price, or at Cayman’s option, the replacement, at no cost to Buyer, of all material that does not meet our specifications.

Said refund or replacement is conditioned on Buyer giving written notice to Cayman within thirty (30) days after arrival of the material at its destination. Failure of Buyer to give said notice within thirty (30) days shall constitute a waiver by Buyer of all claims hereunder with respect to said material.

For further details, please refer to our Warranty and Limitation of Remedy located on our website and in our catalog.

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NOTES

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