MitoCheck® Complex I Activity Assay Kit

Item No. 700930

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

TABLE OF CONTENTS

GENERAL INFORMATION

Materials Supplied

Kit will arrive packaged as a -80°C kit. For best results, remove components and store as stated below.

Item Number	Item	Quantity/Size	Storage
700931	Mitochondrial Complex I Activity Assay Buffer	2 vials/10 ml	-20°C
700018	Ubiquinone Assay Reagent	1 vial/100 µl	-80°C
700932	NADH Assay Reagent	1 vial/500 µg	-20°C
700019	Bovine Heart Mitochondria Assay Reagent	1 vial/100 µl	-80°C
700933	Fatty Acid Free - BSA Assay Reagent	1 vial/250 µl	-20°C
700020	Half Volume 96-Well Clear Plate	1 plate	RT

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

Email: techserv@caymanchem.com

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 a 340 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
4. Mitochondrial Inhibitors - Rotenone, Potassium Cyanide, or Antimycin A
5. 0.1 M NaOH

INTRODUCTION

Background

Complex I (NADH dehydrogenase/NADH:ubiquinone oxidoreductase) is one of the major sites of electron entry into the mitochondrial electron transport chain (ETC). Complex I catalyzes the 2 electron oxidation of NADH followed by the reduction of ubiquinone (Q) to form ubiquinol (QH_2), and ultimately the reduction of the terminal electron acceptor, O_2. During the passage of electrons from NADH to Q, the translocation of four protons (H^+) from the mitochondrial matrix to the intermembrane space occurs, contributing to the chemiosmotic proton gradient, which is required for oxidative phosphorylation.[1] Inhibition of complex I results in severe mitochondrial dysfunction and increased production of reactive oxygen species.[2] Long-term inhibition of complex I has been linked to neurological disorders such as Parkinson's disease, Down's syndrome, and Leigh's syndrome, making it a useful indicator for neurological defects resulting from mitochondrial toxicity.[3-5] This assay is designed so that direct inhibitory effects on complex I can be readily observed. Common inhibitors of complex I activity include rotenone and piericidin.

About This Assay

Cayman's MitoCheck® Complex I Activity Assay allows for the activity of complex I to be determined without the need to isolate mitochondria or pre-incubate with antibodies. The rate of NADH oxidation is measured by a decrease in absorbance at 340 nm and is proportional to the activity of complex I. The rate of NADH oxidation by complex I is fully inhibited by the prototypical complex I inhibitor rotenone (1 µM) (not supplied). For the use of this kit with other types of tissue mitochondria, please see Cayman's MitoCheck® Mitochondrial (Tissue) Isolation Kit (Item No. 701010).

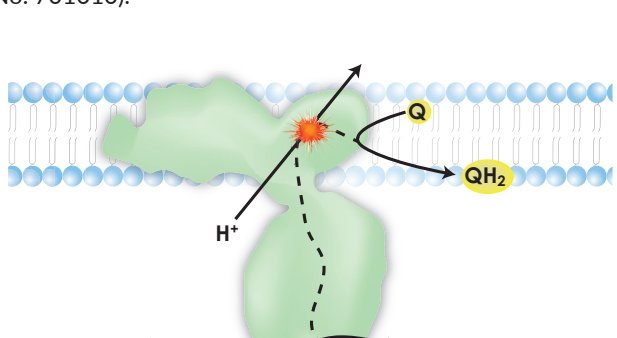


Figure 1. Reaction catalyzed by complex I, as measured in this assay kit.

PRE-ASSAY PREPARATION

Reagent Preparation

All assay reagents, unless listed below, are ready to use as supplied.

1. **Mitochondrial Complex I Activity Assay Buffer - (Item No. 700931)**

   This buffer is ready to use as supplied. It is important that the buffer is warmed to room temperature prior to use. Additionally, vortex well to be sure that any crystals that may have precipitated have dissolved.

2. **NADH Assay Reagent - (Item No. 700932)**

   Each vial contains lyophilized NADH. Dissolve in 155 µl of ultra-pure water. The solution is stable on ice for three hours and should be stored at -20°C once reconstituted (use within two weeks).

3. **Mitochondrial Inhibitors - *(Not Supplied)***

   1. **Potassium Cyanide (KCN)** - KCN should be present to inhibit the ETC (complex IV) and prevent the oxidation of Q. It is important that extreme care is taken when preparing and using KCN. Protocol: In a **ventilated hood**, weigh out 6.5 mg of KCN and dissolve in 1 ml of 0.1 M NaOH to yield a 100 mM stock solution of KCN. **Do not use water or any acidic solvents to make up KCN.** Store stock solution on ice and make fresh less than three hours prior to running this assay. Use appropriate personal protective equipment (PPE).
   2. **Rotenone (Item No. 13995)** - to ensure inhibition of complex I, use concentrations ≥1 µM. This solution can be made up in DMSO or ethanol. If making up in DMSO, avoid freeze/thaws. Use appropriate PPE.
   3. **Antimycin A** *(Optional)* - to ensure inhibition of complex III if the complex IV inhibitor, KCN, is unavailable. Use at concentrations ≥10 µM. Can be made up in DMSO or ethanol. Use appropriate PPE.

ASSAY PROTOCOL

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.

General Information

- The final volume of the assay is 100 µl in all wells.
- It is not necessary to use all the wells on the plate at one time.
- It is recommended that the samples be assayed at least in duplicate (triplicates preferred).
- The assay is performed in the kinetic read mode at 25°C.
- Monitor the absorbance at 340 nm every 30 seconds for 15 minutes.

Performing the Assay

Label two polystyrene tubes as A and B and add the following reagents. *Isolated mitochondria can settle over time, so make sure contents of each tube are well mixed. Store tubes on ice until ready to use. Volumes indicated below are suitable for 20 reactions (or wells). Customer may scale volumes as needed.*

Tube A (1 ml)	Tube B (675 µl)
910 µl of Complex I Activity Assay Buffer	625 µl of Complex I Activity Assay Buffer
20 µl of 100 mM KCN (1 mM) *not supplied*	30 µl of NADH Assay Reagent
50 µl FF-BSA Assay Reagent	20 µl of Ubiquinone Assay Reagent
20 µl Bovine Heart Mitochondria Assay Reagent	

Table 1. Assay preparation

All assays are carried out at 25°C.

1. Add 50 µl of the contents of tube A to each well.
2. Add 20 µl of test compound, positive control, or vehicle diluted in Assay Buffer to each well. Allow for pre-incubation if required.
3. Add 30 µl of the contents of tube B to each well. This should be done quickly as the reaction will start immediately.
4. Immediately place plate in plate reader and measure absorbance at 340 nm (30 second intervals for 15 minutes at 25°C).

ANALYSIS

Calculations

1. Plot data as absorbance (y-axis) *versus* time (x-axis).
2. To determine the reaction rate, calculate the slope for the linear portion of the curve.
3. Determine % activity relative to the vehicle control using the equation indicated below.
4. To determine an IC_{50} value for each compound, plot the slope as a function of test compound concentration.

$$\text{Complex I Activity (\%)} = \left[\frac{\text{Rate of Sample wells}}{\text{Rate of Vehicle Control}}\right] \times 100$$

Performance Characteristics

The data shown below are an example of data obtained with this kit. Your results will not be identical to these.

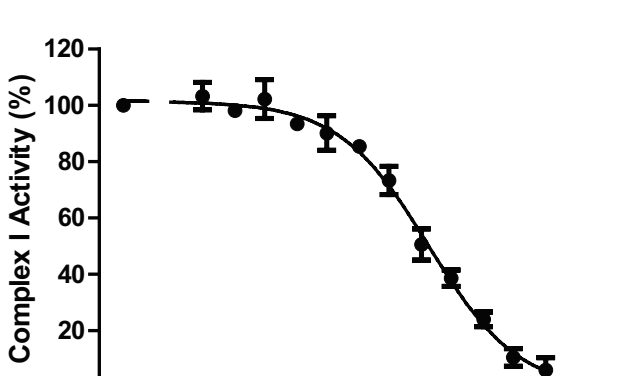


Figure 3. A typical concentration response curve for inhibition of complex I activity by rotenone (IC_{50} = 131 nM). "Veh." represents compound vehicle control.

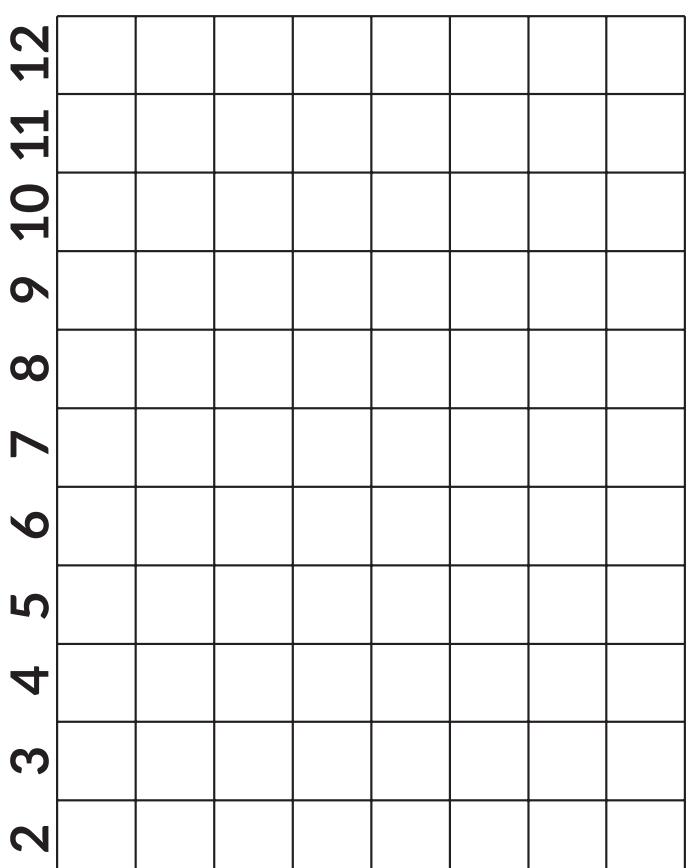
RESOURCES

Troubleshooting

Problem	Possible Causes	Recommended Solutions
Erratic values; dispersion of duplicates/triplicates	A. Poor pipetting/technique B. Bubble in the well(s) C. Poor test compound solubility	A. Be careful not to splash the contents of the wells B. Carefully tap the side of the plate with your finger to remove bubbles C. Test solubility with assay buffer
No activity was detected in test compound wells	Test compound is a potent inhibitor	Check vehicle controls to be sure complex I is active
Test compound absorbance is above saturating positive control (*i.e.*, rotenone) absorbance	Test compound absorbs at 340 nm	Determine absorbance of compounds in the absence of NADH; subtract this value from all wells containing test compound

References

1. Brown, G.C. and Brand, M.D. Proton/electron stoichiometry of mitochondrial complex I estimated from the equilibrium thermodynamic force ratio. *Biochem. J.* **252(2)**, 473-479 (1988).
2. Li, N., Ragheb, K., Lawler, G., *et al.* Mitochondrial complex I inhibitor rotenone induces apoptosis through enhancing mitochondrial reactive oxygen species production. *J. Biol. Chem.* **278(10)**, 8516-8525 (2003).
3. Sherer, T.B., Betarbet, R., Testa, C.M., *et al.* Mechanism of toxicity in rotenone models of Parkinson's disease. *J. Neurosci.* **23(34)**, 10756-10764 (2003).
4. Papa, S. and De Rasmo, D. Complex I deficiencies in neurological disorders. *Trends Mol. Med.* **19(1)**, 61-69 (2013).
5. Janetzky, B., Hauck, S., Youdim, M.B.H., *et al.* Unaltered aconitase activity, but decreased complex I activity in substantia nigra pars compacta of patients with Parkinson's disease. *Neurosci. Lett.* **169(1-2)**, 126-129 (1994).



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.