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Product Information

Mitochondrial Complex I Activity Assay Kit

Catalog Number **MAK359**
Storage Temperature –20 °C

TECHNICAL BULLETIN

Product Description

Mitochondrial Complex I or NADH:ubiquinone oxidoreductase is the first and the largest complex of the electron transport chain located in the mitochondrial membrane. It oxidizes NADH to NAD^+ and transfers an electron to ubiquinone (also present in the inner mitochondrial membrane) converting it to ubiquinol. During this process, it transports protons across the inner mitochondrial membrane, helping to develop an electrochemical gradient. This process is very important for cellular respiration and adverse effects on Complex I activity can compromise mitochondrial respiration, which further leads to cellular stress.

The Mitochondrial Complex I Assay Kit is a fast and reliable method to determine the activity of Complex I in isolated mitochondria. It is useful for respiration studies in isolated mitochondria and may be used to study effects of toxicants, drugs, and other environmental conditions on mitochondrial complex I activity.

This kit uses decylubiquinone, an analog of ubiquinone, as an electron acceptor that gets converted to decylubiquinol through the catalytic activity of Complex I. The Complex I dye absorbs light at 600 nm (A_{600}) in its oxidized form. It is used as a terminal electron acceptor, accepting electrons from decylubiquinol. Complex I activity is determined colorimetrically by recording the change in absorbance (A_{600}) of the reduced Complex I dye. Specific Complex I activity is obtained by subtracting the activity in presence of Complex I inhibitor rotenone from total activity. This kit can detect as low as 0.1 mU/well and is linear up to 7 mU/well.

The kit is suitable for the measurement of Mitochondrial Complex I activity in isolated mitochondria.

Components

The kit is sufficient for 100 colorimetric assays in 96 well plates.

Complex I Assay Buffer Catalog Number MAK359A	25 mL
NADH Catalog Number MAK359B	1 vial
Decylubiquinone Catalog Number MAK359C	1 vial
Complex I Dye Catalog Number MAK359D	1 vial
Complex I Inhibitor Rotenone Catalog Number MAK359E	100 µL

Reagents and Equipment Required but Not Provided.

- Pipetting devices and accessories (e.g., multichannel pipettor)
- Spectrophotometric multiwell plate reader
- Dimethyl Sulfoxide (DMSO), anhydrous (Catalog Number 276855)
- Mitochondria Isolation Kit
- Corning® 96 Well Half-Area Microplate (Catalog Number CLS3697)
- Bicinchoninic Acid Kit for Protein Determination (Catalog Number BCA1)

Precautions and Disclaimer

For R&D use only. Not for drug, household, or other uses. Please consult the Safety Data Sheet for information regarding hazards and safe handling practices.

Storage/Stability
The kit is shipped on wet ice. Store components at –20 °C, protected from light. Complex I Assay Buffer may be stored at room temperature. Briefly centrifuge small vials prior to opening. Keep all reagents on ice while performing the assay.

Preparation Instructions.
Reagent Preparation
Complex I Assay Buffer: Warm to room temperature prior to use

NADH: Reconstitute the vial with 56 µL of ultrapure water to obtain a 100× stock solution. Pipette up and down a few times to dissolve completely. The solution should be clear and must be protected from light. Centrifuge briefly after mixing and use the same day. Stock solution should be frozen at –20 °C.

Decylubiquinone: Reconstitute with 310 µL of DMSO to obtain a 2× stock solution. Centrifuge briefly after dissolving. Keep on ice protected from light while running the assay. Aliquot and store at –20 °C.

Complex I Dye (10×): Reconstitute with 450 µL of Complex I Assay Buffer to obtain a 10× solution (10 mM). Centrifuge briefly after mixing. Aliquot and store at –20 °C.

Complex I Inhibitor Rotenone: Aliquot and store at –20 °C.

Procedure
Sample Preparation
1. Isolate mitochondria using preferred protocol.
2. Estimate the protein concentration of isolated mitochondrial samples. Mitochondrial protein concentration should be at least 500 µg/ml. 1-5 µg will be needed per reaction.
3. Isolated mitochondria should be aliquoted and stored at –80 °C unless being used for the assay immediately. Avoid repeated freeze thaw cycles.
4. Mitochondria should be placed on ice during the course of the assay. Assays should be performed within 3 hours of isolation.
5. Different dilutions of the mitochondrial sample should be tested to make sure that the activity falls in the linear range of the assay. Dilutions should be prepared in Complex I Assay Buffer immediately before performing the assay.

Standard Curve Preparation
Use Complex I Dye to prepare the Standard Curve. Prepare 1× Complex I Dye working solution (1 mM) by diluting 10 µL of 10 mM Complex I Dye with 90 µL of Complex I Assay Buffer, mix well. Prepare Dye Standards in desired wells of a clear 96 well half-area plate according to Table 1. Mix well. Measure the absorbance (A_{600}) at 600 nm (end-point).

Table 1.
Preparation of Dye Standards

Well	1 mM Premix	Complex I Assay Buffer	Dye (nmol/well)
1	0 µL	100 µL	0
2	2 µL	98 µL	2
3	4 µL	96 µL	4
4	6 µL	94 µL	6
5	8 µL	92 µL	8
6	10 µL	90 µL	10

Reaction Mix
Prepare 1× decylubiquinone solution by diluting the 2× stock solution with DMSO 2-fold. Prepare 1× Complex I Dye working solution by diluting 10× Complex I Dye 10-fold with Complex I Assay Buffer, mix well. Mix enough reagents for the number of assays to be performed. Sample will be added after the reaction mix. Prepare 70 µL of Reaction Mix for background control and 68 µL of Reaction Mix per reaction for the "Sample Mix" and "Sample + Inhibitor Mix" per well according to Table 2.

Table 2.
Preparation of Reaction Mixes

Reagent	Background Control	Sample Mix	Sample + Inhibitor Mix
Complex I Assay Buffer	59 µL	57 µL	56 µL
Decylubiquinone (1x)	2 µL	2 µL	2 µL
Complex I Dye (1x)	9 µL	9 µL	9 µL
Complex I Inhibitor Rotenone	–	–	1 µL

Add the reaction mixes to the corresponding wells of a clear bottom 96 well half-area plate.

Mitochondrial Sample addition and measurement

1. Set plate reader to 600 nm on kinetic mode at 30 second intervals.
2. Add 2 μL of mitochondrial samples (1 to 5 μg) to wells containing "Sample Mix" and "Sample + Inhibitor Mix", mix well.
3. Prepare NADH 1× working solution by diluting with Complex I Assay Buffer (i.e 10 μL of NADH 100× plus 990 μL of Complex I Assay Buffer). Keep NADH 1× working solution on ice.
4. Add 30 μL of 1× NADH to each well using a multichannel pipette. Total volume in each will be 100 μL.
5. Read plate **immediately** at 600 nm for 5 minutes at room temperature.

Results

1. Use the standard curve to obtain the amount of oxidized Complex I Dye in sample wells.
2. Since the assay is based on reduction of the Complex I Dye, amount of reduced Complex I Dye per well can be obtained by subtracting the amount of oxidized Complex I Dye (as read form standard curve) from total Complex I Dye added to the assay (9 nmol/well).
3. Find the concentration of reduced Complex I Dye between time points t_1 and t_2.
4. Calculate Δ[reduced Complex I Dye concentration] between times t_1 and t_2.
5. Apply the following equation to obtain activity of complex I.

Sample Complex I Activity (mUnits/μg) =

$$\frac{\Delta[\text{reduced Complex I Dye concentration}] \times D}{(\Delta t \times p)}$$

where:

Δ[reduced Complex I Dye concentration] = Change in reduced Complex I Dye concentration during Δt

$\Delta t = t_2 - t_1$ (minutes)

p = mitochondrial protein (μg)

D = the sample dilution factor (D = 1 for undiluted samples).

Net Complex I Activity in sample=

Activity in reaction without rotenone – Activity in reaction with rotenone

Unit Definition

One unit of Complex I is the amount of enzyme that will cause reduction of 1.0 μmol of the dye per minute at pH 7.4 at room temperature.

Figure 1.
Typical Oxidized Complex I Dye Standard Curve.

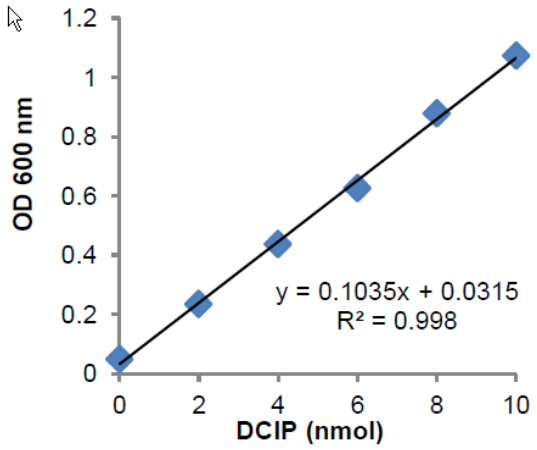


Figure 2.
A_{600} with Varying Concentrations of Bovine Heart Mitochondria Obtained Commercially.

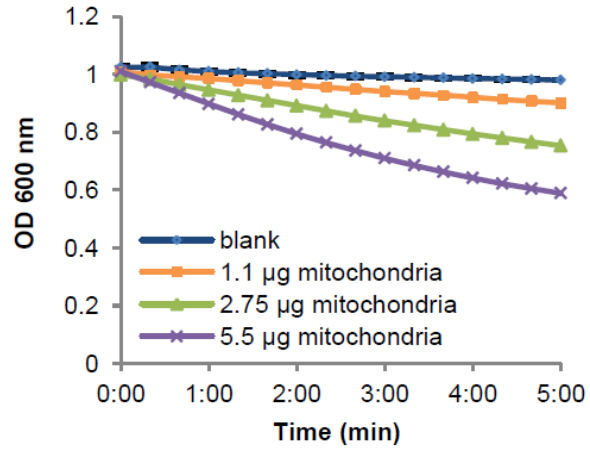
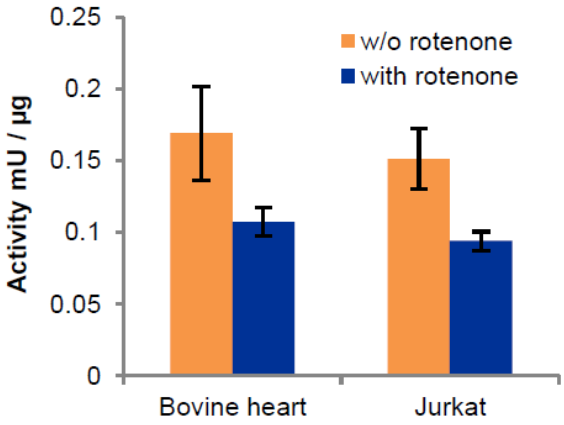


Figure 3.
Complex I Activity in isolated Bovine heart mitochondria and jurkat cell mitochondria with and without Complex I inhibitor rotenone.



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