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Product Information

Mitochondrial Complex III Activity Assay Kit

Catalog Number **MAK360**
Storage Temperature –20 °C

TECHNICAL BULLETIN

Product Description

Mitochondrial Complex III or Ubiquinol-Cytochrome c reductase is the third complex of the electron transport chain located in the mitochondrial membrane. It is present in the mitochondria of all aerobic eukaryotes as well as in the inner membranes of most bacteria. It transfers electrons from CoQH (reduced coenzyme Q or ubiquinol) to cytochrome c, resulting in the reduction of cytochrome c. This reduced cytochrome c is a substrate for Complex IV of the electron transport chain.

The Mitochondrial Complex III Activity Assay is based on the reduction of cytochrome c through the activity of Complex III. The absorbance (A_{550}) of reduced cytochrome c can be measured at 550 nm. It is a fast and reliable method to determine the activity of Complex III in isolated mitochondria. The kit can detect as low as 10 mU/mL and is linear up to 30 mU/mL.

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

Components

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

Complex III Assay Buffer Catalog Number MAK360A	25 mL
Cytochrome c Catalog Number MAK360B	4 × 1 vial
Complex III Inhibitor Antimycin A Catalog Number MAK360C	220 µL
1 M DTT Catalog Number MAK360D	1 mL

Reagents and Equipment Required but Not Provided.

- Pipetting devices and accessories (e.g., multichannel pipettor)
- Spectrophotometric multiwell plate reader
- Mitochondria Isolation Kit
- Corning® 96 Well Half-Area Microplate (Catalog Number CLS3697)
- Bradford Reagent (Catalog Number B6916)
- Dimethyl Sulfoxide (DMSO), anhydrous (Catalog Number 276855)

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. Complex III Assay Buffer may be stored at 4 °C. Briefly centrifuge small vials prior to opening.

Preparation Instructions.

Reagent Preparation

Complex III Assay Buffer: Warm to room temperature prior to use

Cytochrome c: Reconstitute one vial at a time with 175 µL of Complex III Assay Buffer to obtain a 2 mM solution. Centrifuge briefly after mixing. One reconstituted vial is sufficient for 25 reactions. Store at –20 °C while not in use. The reconstituted vial is stable for at least one month.

Complex III Inhibitor Antimycin A: Aliquot and store at –20 °C.

1 M DTT: Thaw prior to use.

Procedure

Sample Preparation

1. Isolate mitochondria from cultured cells or tissue using preferred method.
2. Estimate the protein concentration of isolated mitochondrial samples using Bradford Reagent.
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.
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 III Assay Buffer immediately before performing the assay.

Standard Curve Preparation

1. Prepare a 750 mM DTT solution by mixing 1 M DTT solution and Assay buffer in a ratio of 3:1.
2. Prepare Cytochrome c Standards in desired wells of a clear 96 well half-area plate according to Table 1.

Table 1.
Preparation of Cytochrome c Standards

Well	2 mM Premix	Complex III Assay Buffer	Cytochrome c (nmol/well)
1	0 µL	23 µL	0
2	2 µL	21 µL	4
3	4 µL	19 µL	8
4	6 µL	17 µL	12
5	8 µL	15 µL	16
6	10 µL	13 µL	20

3. Mix well.
4. Add 2 µL of the prepared 750 mM DTT solution in each well in order to completely reduce cytochrome c.
5. Mix well and incubate at room temperature for 5 minutes.
6. Measure the absorbance (A_{550}) at 550 nm (end-point).

Reaction Mix

Mix enough reagents for the number of assays to be performed. For each well, prepare reaction mix according to Table 2.

Table 2.
Preparation of Reaction Mixes

Reagent	Background Control	Sample Mix	Sample Mix with Complex III Inhibitor
Complex III Assay Buffer	17 µL	15 µL	15 µL
Inhibitor Antimycin A	–	–	2 µL
DMSO	2 µL	2 µL	–

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

Mitochondrial Sample addition and measurement

1. Set plate reader to 550 nm on kinetic mode at 30 second intervals.
2. Add 1 to 2 µL mitochondrial samples (1.5 to 10 µg protein) to wells containing “Sample Mix” and “Sample Mix with Complex III Inhibitor”.
3. Mix well.
4. Add 6 µL of Cytochrome c (substrate).
5. Read plate **immediately** at 550 nm for 10 minutes at room temperature.

Results

1. Complex III specific activity may be calculated by comparing sample A_{550} values (after subtraction of background control) to the reduced cytochrome c standard curve.
2. Calculate the concentration of reduced cytochrome c, at time t_1 and t_2 by reading off the standard curve.
3. Calculate Δ[cytochrome c] between time t_1 and t_2.
4. Apply the following equation to obtain activity of Complex III.

Sample Complex III Activity (Units/µg) =

$$\frac{\Delta C}{(\Delta t \times p)} \times D$$

where:

ΔC = Change in reduced cytochrome c 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 III Activity in sample =

Activity in reaction without Antimycin A – Activity in reaction with Antimycin A

Unit Definition

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

Figure 1.
Typical DTT Reduced Cytochrome c Standard Curve

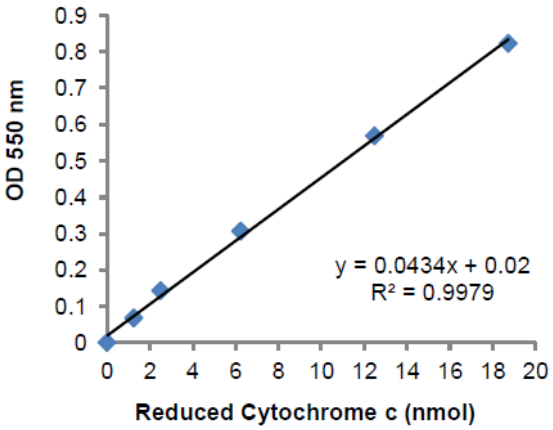


Figure 2.
Complex III Activity with Increasing Amounts of Bovine Heart Mitochondria

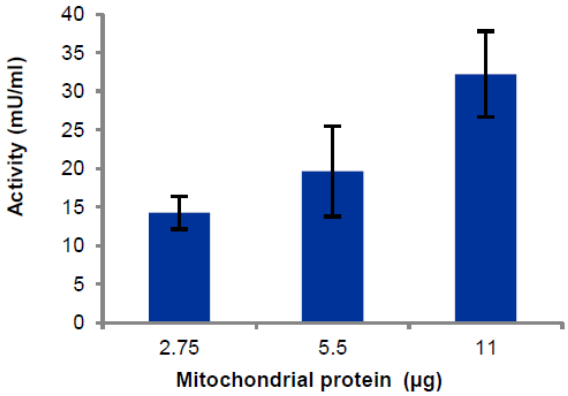
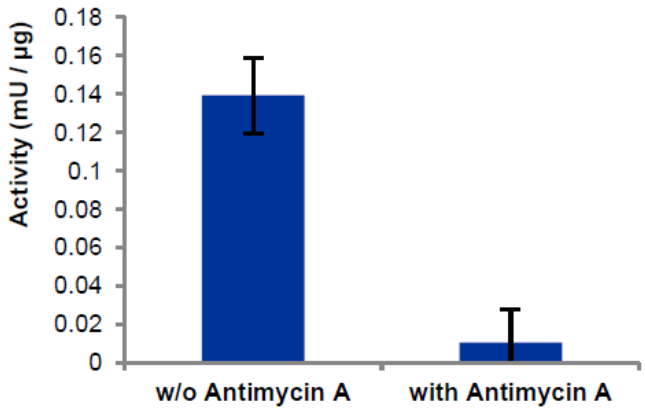


Figure 3.
Activity of Complex III in isolated bovine heart mitochondria in absence and presence of Antimycin A (units/µg protein).



Assays were performed following the kit procedure.

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