ab287844 – Mitochondrial Complex III Activity Assay Kit

For in vitro quantitative determination of the activity of complex III in isolated mitochondria.
For research use only - not intended for diagnostic use.

For overview, typical data and additional information please visit:
http://www.abcam.com/ab287844

Storage and Stability

An unopened kit can be stored at -20°C for 6 months.

Materials Supplied

Item	Quantity	Storage Condition
Complex III Assay Buffer	25 mL	-20°C
Oxidized Cytochrome c	4 X 1 vials	-20°C
Antimycin A	200 µL	-20°C
DTT IV	1 mL	-20°C
96-Well Half Area Plate	1 unit	-20°C

Materials Required, Not Supplied

These materials are not included in the kit, but will be required to successfully utilize this assay:

- Multi-well spectrophotometer capable of reading absorbance in kinetic mode
- DMSO (anhydrous)
- Deionized water

Reagent Preparation

- Before using the kit, spin the tubes prior to opening.

Complex III Assay Buffer: Store at 4°C. Bring to RT before use.

Oxidized Cytochrome c: Reconstitute one vial at a time with 175 µl Complex III Assay Buffer to obtain 2 mM solution. Once reconstituted, one vial is enough for 25 reactions. Centrifuge briefly after mixing. Store at -20°C. The reconstituted vial stored at -20°C should be stable for at least one month.

Antimycin A: Aliquot and store at -20°C.

DTT IV: Thaw before use.

Δ Note: *Antimycin A and DTT IV are stable for at least 3 months at* -20°C.

Sample Preparation

Isolate mitochondria from cultured cells or tissue using preferred procedure or kit.

- Estimate the protein concentration of isolated mitochondrial samples using Bradford assay.
- Isolated mitochondria should be stored at -80°C unless being used for the assay immediately. Avoid freeze-thaw cycles.
- 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.

Δ Note: *Mitochondria should be placed on ice during the assay.*

Assay Protocol

Reduced Cytochrome c Standard Curve

1. Prepare 750 mM DTT IV solution by mixing 1 M DTT IV solution and Assay Buffer in a ratio 3:1.
2. Add 0, 2, 4, 6, 8 and 10 µl of 2.0 mM Oxidized Cytochrome c standard into a series of wells in the provided 96-Well Half Area Plate to generate 0, 4, 8, 12, 16, and 20 nmol/well of Oxidized Cytochrome c.
3. Adjust the volume to 23 µl/well with Complex III Assay Buffer.
4. Add 2 µl of the prepared 750 mM DTT IV solution in each well in order to completely reduce Oxidized Cytochrome c.
5. Incubate at RT for 10 min on a plate shaker set to 200 rpm.
6. Measure the absorbance at 550 nm (end point).

Reaction Mix

- Mix enough reagents for the number of assays to be performed. For each well, prepare reaction mix containing:

Item	Background Control Well	Sample Mix Well	Sample Mix with Antimycin A
Assay Buffer	17 µl	15 µl	15 µl
Antimycin A	-	-	2 µl
DMSO	2 µl	2 µl	-

- Add the reaction mix to wells of the clear bottom 96-well half area plate which is provided with the kit.

Mitochondrial Sample

1. Add 1 to 2 µl mitochondrial samples (1.5 to 10 µg protein) to wells containing "Sample Mix" and "Sample Mix with Antimycin A". Mix well.
2. Add 6 µl Oxidized Cytochrome c (substrate) and read immediately.

Δ Note: *Have the 96-Well Half Area Plate/plate reader ready at 550 nm on kinetic mode.*

Measurement

- Immediately start recording absorbance at 550 nm, at 30 second intervals for up to 10 minutes at room temperature.

Calculation

- Complex III specific activity may be calculated by comparing sample OD values (after subtraction of background control) to the reduced Oxidized Cytochrome c standard curve.
- Calculate the concentration of reduced Oxidized Cytochrome c, at time t1 and t2 by reading off the standard curve.
- Calculate Δ [cytochrome c] between time t1 and t2. Apply the following equation to obtain activity of complex III:

$$\text{Complex III specific activity} = \frac{\Delta C}{\Delta t \times p} \times D \text{ (Units/µg)}$$

Where:

- ΔC is the change in reduced cytochrome c concentration during Δt
- Δt is the time duration between t1 and t2, equal to: t2 – t1 (min)
- p is the mitochondrial protein sample (µg)
- D is the dilution factor

- Use the following equation to calculate net activity:

$$\text{Net Complex III Activity in sample} = \textit{Activity in reaction without Antimycin A} - \textit{Activity in reaction with Antimycin A}$$

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

Technical Support

For all technical or commercial enquiries please go to:

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