Tribioscience

Mitochondria Complex I Activity Colorimetric Assay

(Catalog: TBS2017, 100 Assays, Store at -20ºC)

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.

This kit uses decylubiquinone, an analog of ubiquinone, as an electron acceptor that gets converted to decylubiquinol through the catalytic activity of Complex I. Complex I activity is determined by recording the change in absorbance (A600) 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.

Applications

- Complex I Activity.

Kit Contents for 100 Test:

Name	Part Size
Complex I Assay Buffer	12 mL
NADH (100x)	50 µL
Decylubiquinone (2x)	0.1 mL
Complex I Dye (10 mM)	0.25 mL
Complex I Inhibitor Rotenone	0.1 mL

Storage conditions: Store the Reagent at –20°C protected from light. Shelf life: 12 months.

Procedures

Mitochondria Isolation

Mitochondria isolation is performed with suitable approach. We recommend Tribioscience Mitochondria Isolation kit (TBS2016) to isolation mitochondria from cells or tissues. The whole procedure is operated on ice.

Standard Curve Preparation

Use Complex I Dye to prepare the Standard Curve.

1. 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.
2. Prepare Dye Standards in desired wells of a clear 96 well plate according to Table 1. Total volume 100 µL/well. Mix well.

Table 1: Standard Curve Preparation

Well	1 mM Premix Dye	Assay Buffer	Dye (nM)/well
1	0	100	0
2	1	99	1
3	2	98	2
4	4	96	4
5	6	94	6
6	8	92	8
7	10	90	10

Reaction Mix

3. Prepare 1× decylubiquinone solution by diluting the 2× stock solution with Ethanol 2-fold.
4. Prepare 1× Complex I Dye working solution by diluting 10× Complex I Dye 10-fold with Complex I Assay Buffer, mix well.
5. Mix enough reagents for the number of assays to be performed. Sample will be added after the reaction mix.
6. 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: Reaction Mix Preparation

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

7. Add the reaction mix to the indicated wells.
8. Set up the plate reader at 600 nm on kinetic mode at 30 second intervals.
9. Add 2 µL of mitochondrial samples (3 to 10 µg) to wells containing "Sample Mix" and "Sample + Inhibitor Mix", mix well.
10. 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.
11. Add 30 µL of 1× NADH to each well using a multichannel pipette. Total volume in each well will be 100 µL (Note: Do not add it into Standard wells).
12. Read plate immediately at 600 nm for 10 minutes at room temperature.

Data Analysis

13. Use the standard curve to obtain the amount of oxidized Complex I Dye in sample wells. Fig. 1 is an example of typical standard curve.
14. 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 from standard curve) from total Complex I Dye added to the assay (9 nM/well).
15. Find the amount of reduced Complex I Dye (nM) between time points t1 and t2.
16. Calculate Δ[reduced Complex I Dye amount (nM)] between times t1 and t2.
17. Apply the following equation to obtain activity of complex I:

Sample Complex I Activity (nM/min/µg) = Δ[reduced Complex I Dye amount] × D/ (Δt × p)

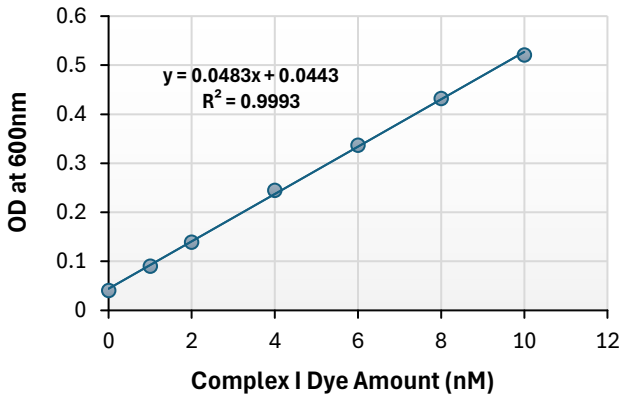
where: Δ[reduced Complex I Dye amount] = Change in reduced Complex I Dye amount during Δt (nM).

Δt = t2 – t1 (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.

Fig.1 Standard Curve of Complex I Dye



Relative Products

Mitochondria Isolation Kit (TBS2016)
Mitochondrial Membrane Potential Assay (TBS2049)
Cytochrome C Oxidase (Complex IV) Activity Assay (TBS2115)
Cytochrome C reductase Activity Assay (TBS2116)
NAD/NADH Colorimetric Assay (TBS2029)
Resazurin Cell Viability Kit (TBS2001)
CCK-8 Cell Viability Assay (TBS2022)
ATP Colorimetric/Fluorometric Assay Kit (TBS2010)
ADP Colorimetric/Fluorometric Assay Kit (TBS2020)
Caspase-3 Colorimetric Assay kit (TBS2030)

This product is for research only.