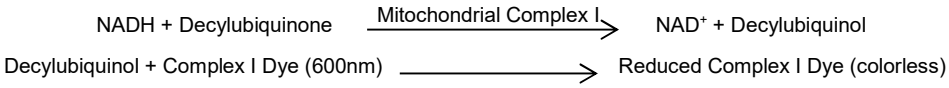
Mitochondrial Complex I Activity Colorimetric Assay Kit (BN01128)

(Catalog #BN01128; 100 assays; Store at -20°C)

I. Introduction:

Mitochondrial Complex I or NADH:ubiquinone oxidoreductase (EC 1.6.5.3) 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. Assay Genie's 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 that absorbs light at 600 nm in its oxidized form is used as a terminal electron acceptor that accepts electrons from decylubiquinol. Complex I activity is determined colorimetrically by recording the change in absorbance of reduced Complex I dye at 600 nm. 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.



II. Application:

- Measurement of Mitochondrial Complex I enzymatic activity in isolated mitochondria.

III. Sample Type

- Isolated Mitochondria

IV. Kit Contents:

Components	BN01128	Cap Code	Part Number
Complex I Assay Buffer	25 ml	WM	BN01128-1
NADH	1 vial	Yellow	BN01128-2
Decylubiquinone	1 vial	Blue	BN01128-3
Complex I Dye	1 vial	Red	BN01128-4
Complex I Inhibitor Rotenone	100 µl	Green	BN01128-5
Clear 96-well half area plate	1 plate	-	BN01128-6

V. User Supplied Reagents and Equipment:

- DMSO
- Deionized water
- Multi-well Spectrophotometer capable of reading absorbance in kinetic mode

VI. Storage Conditions and Reagent Preparation:

Store kit at –20°C, protected from light. Complex I Assay Buffer may be stored at RT. Warm all buffers to room temperature before use. Briefly centrifuge all small vials prior to opening. Keep all reagents on ice while performing the assay.

- **NADH:** Reconstitute the vial with 56 µl deionized water to obtain a 100 X stock solution. Pipette few times to dissolve completely. The solution should be clear and must be protected from light. Centrifuge briefly after mixing. Protect from light and use the same day. Stock solution should be frozen at -20ºC.
- **Decylubiquinone:** Reconstitute with 310 µl DMSO to get 2X stock solution. Centrifuge briefly after dissolving. Keep on ice protected from light while running the assay. Aliquot and store at -20ºC.
- **Complex I Dye:** Reconstitute with 450 µl Complex I Assay Buffer to obtain 10 X solution (10 mM). Centrifuge briefly after mixing. Aliquot and store at -20ºC.
- **Complex I Inhibitor Rotenone**: Aliquot and store at -20ºC.

  Note: NADH, Decylubiquinone, Complex I Dye and rotenone stock solutions, should be stable for at least three months at -20ºC.

VII. Mitochondrial Complex I Activity Assay Protocol:

1. **Sample Preparation:** Isolate mitochondria using preferred protocol**.** We recommend Mitochondria Isolation Kit for Tissue & Cultured Cells or Yeast Mitochondria Isolation Kit. 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*.

   Notes:

   a. Isolated mitochondria should be aliquoted and stored at -80ºC unless being used for the assay immediately. Avoid repeated freeze thaw cycles.

   b. Mitochondria should be placed on ice during the course of the assay. Assays should be performed within 2 – 3 hours.

   c. Different dilutions of the mitochondrial sample should be tested to make sure that the activity falls in the linear range of the assay.

d. Dilutions should be prepared in Complex I Assay Buffer immediately before performing the assay.

2. **Standard Curve Generation:** Use Complex I Dye to prepare Standard Curve**.** Prepare 1 X Complex I Dye working solution (1 mM) by diluting the Complex I Dye stock solution with Complex I Assay Buffer (i.e 10 µl 10 mM Complex I dye + 90 µl Complex I Assay Buffer). Add 0, 2, 4, 6, 8 and 10 µl of 1 mM Complex I Dye solution into a series of wells in the provided 96-well half area plate to generate 0, 2, 4, 6, 8 and 10 nmol / well of the dye. Adjust the volume to 100 µl/well with Complex I Assay Buffer. Mix well. Measure the absorbance at 600 nm (end point).
3. **Reaction Mix**: Prepare 1 X decylubiquinone solution by diluting the 2X stock solution with DMSO in 1:1 ratio. Mix enough reagents for the number of assays to be performed. *Sample will be added after the reaction mix*. Prepare 70 µl reaction mix for background control and 68 µl reaction mix per reaction for the "Sample Mix" and "Sample + Inhibitor Mix" per well as follows:

	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
Inhibitor rotenone	-	-	1 µl

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

4. **Mitochondrial Sample:** Add 2 µl mitochondrial samples (1 to 5 µg) to wells containing "Sample Mix" and "Sample + Inhibitor Mix". Mix well. Prepare NADH 1 X working solution by diluting with Complex I Assay Buffer (i.e 10 µl NADH 100 X + 990 µl Complex I Assay Buffer). Keep NADH 1 X working solution on ice. Add 30 µl of 1X NADH to each well using a multi-channel pipette to make up 100 µl total volume. *Read immediately*.

Note: Have the plate reader ready at 600 nm on kinetic mode.

5. **Measurement:** Set the plate reader at OD_{600nm} in kinetic mode at 30 second intervals. *Read immediately after the addition of 1X NADH.* Absorbance may be recorded for up to 5 minutes at RT.
6. **Calculation:** Use the standard curve to obtain the amount of oxidized Complex I Dye in sample wells. 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). Find the concentration of reduced Complex I Dye between time points t1 and t2. Calculate Δ[reduced Complex I Dye concentration] between times t1 and t2. Apply the following equation to obtain activity of complex I.

Sample Complex I Activity = Δ [reduced Complex I Dye concentration] / (Δt x p) x D (mUnits / µg)

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

Δt = t2 – t1 (min)

p = mitochondrial protein (µg)

D = dilution factor

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 min at pH 7.4 at room temperature.

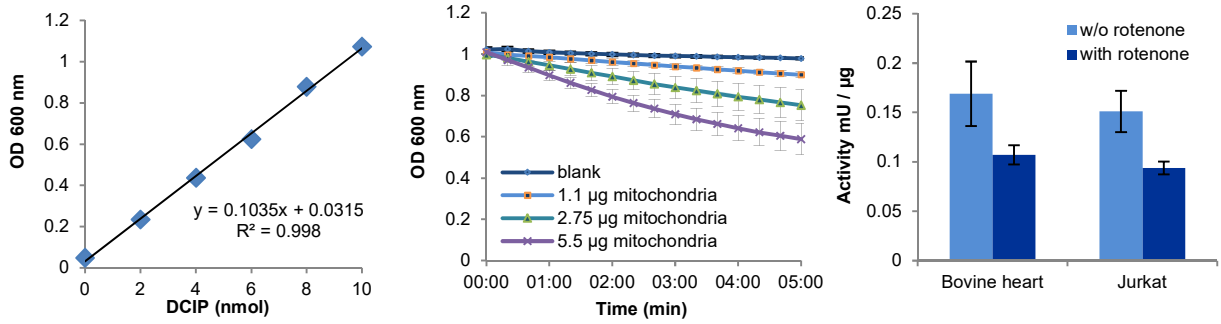


Figure: (a) Standard curve for oxidized Complex I dye. (b) OD_{600} with varying concentrations of Bovine heart mitochondria obtained commercially. (c) Complex I Activity in isolated Bovine heart mitochondria (obtained commercially) and jurkat cell mitochondria (isolated using BV cat # K288-50) with and without Complex I inhibitor rotenone.

FOR RESEARCH USE ONLY! Not to be used on humans.