CREATIVE BIOMART®
Assay Kit

Mit Complex II Activity Assay Kit

Product Information

Cat.No. Kit-0246

Product Overview

Complex II Activity Assay allows for the activity of complex II to be determined without the need to isolate mitochondria or pre-incubate with antibodies. As complex II oxidizes succinate, electrons are passed to an analog of ubiquinone and then on to DCPIP, which, when oxidized, absorbs in the 600 nm range. The absorbance of DCPIP will decrease upon reduction. Complex II activity is measured as a decrease in absorbance at 600 nm over time. To prevent interference from other ETC complexes, rotenone (1 μ M), antimycin A (10 μM), and potassium cyanide (2 mM) are present as inhibitors (not supplied).

Storage

at -80°C

Shipping

Dry ice

Size

96 wells

Kit Components

Mitochondrial Complex II Activity Assay Buffer: 2 vials/10 ml, -20°C;
Ubiquinone Assay Reagent: 1 vial/100 µl, -80°C;
DCPIP Assay Reagent: 1 vial/700 µg, -20°C;
Bovine Heart Mitochondria Assay Reagent: 1 vial/100 µl, -80°C;
Succinate Assay Reagent: 1 vial/100 µl, -20°C;
Half Volume 96-Well Clear Plate: 1 plate, Room temperature

Materials Required but Not Supplied

1. A plate reader capable of measuring absorbance a 600 nm at 30 second intervals
2. Adjustable and multichannel pipettes

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3. A source of pure water; glass distilled water or HPLC-grade water is acceptable
4. Mitochondrial Inhibitors - Rotenone, TTFA, Potassium Cyanide, or Antimycin A
5. 0.1 M NaOH

Technical Notes

- Use different tips to pipette each reagent.
- Avoid introducing bubbles into the well.
- Do not expose the pipette tip to the reagent(s) already in the well.
- The final volume of the assay is 100 µl in all wells.
- It is not necessary to use all the wells on the plate at one time.
- It is recommended that the samples be assayed at least in duplicate (triplicates preferred).
- The assay is performed in the kinetic read mode at 25°C.
- Monitor the absorbance at 600 nm every 30 seconds for 15 minutes.

Preparation

All assay reagents, unless listed below, are ready to use as supplied.
1. Mitochondrial Complex II Activity Assay Buffer
This buffer is ready to use as supplied. It is important that the buffer is warmed to room temperature prior to use. Additionally, vortex well to be sure that any crystals that may have precipitated have dissolved.
2. Mitochondrial Inhibitors - (Not Supplied)
2.1. Potassium Cyanide (KCN) - KCN should be present to inhibit the ETC (complex IV) and prevent the oxidation of Q. It is important that extreme care is taken when preparing and using KCN. Protocol: In a ventilated hood, weigh out 6.5 mg of KCN and dissolve in 1 ml of 0.1 M NaOH to yield a 100 mM stock solution of KCN. Do not use water or any acidic solvents to make up KCN. Store stock solution on ice and make fresh less than three hours prior to running this assay. Use appropriate personal protective equipment (PPE).
2.2. Rotenone - to ensure inhibition of complex I, use concentrations ≥1 µM. Rotenone can be made up in DMSO or ethanol. If making up in DMSO, avoid freeze/thaws. Use appropriate PPE.
2.3. Antimycin A - to ensure inhibition of complex III, use concentrations ≥10 µM. Can be made up in DMSO or ethanol. Use appropriate PPE.

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2.4. 2-Thenoyltrifluoroacetone (TTFA) - to ensure inhibition of complex II, use concentrations ≥1 mM. TTFA can be made up in DMSO or ethanol. Use appropriate PPE.

Assay Protocol

Label two polystyrene tubes as A and B and add the following reagents. Isolated mitochondria can settle over time, so make sure contents of each tube are well mixed. Store tubes on ice until ready to use. Volumes indicated below are suitable for 20 reactions (or wells). Customer may scale volumes as needed.
Tube A (1 ml) Tube B (675 µl)
956 µl of Complex II Assay Buffer 487 µl of Complex II Assay Buffer
20 µl Bovine Heart Mitochondria Assay Regent 8 µl of Succinate Assay Reagent
2 µl of 1 mM Rotenone *not supplied* 20 µl of Ubiquinone Assay Reagent
20 µl of 100 mM KCN (1 mM) *not supplied* 160 µl of DCPIP Assay Reagent
2 µl of 10 mM Antimycin A *not supplied*
Table 1. Assay preparation

All assays are carried out at 25°C.
1. Add 50 µl of the contents of tube A to each well.
2. Add 20 µl of test compound, positive control, or vehicle to each well. Allow for pre-incubation if required.
3. Add 30 µl of the contents of tube B to each well.
4. Incubate five minutes at 25°C, then place plate in plate reader and measure absorbance at 600 nm (30 second intervals for 15 minutes at 25°C).

Analysis

1. Plot data as absorbance (y-axis) versus time (x-axis).
2. To determine the reaction rate, calculate the slope for the linear portion of the curve.
3. Determine % activity relative to the vehicle control using the equation indicated below.
4. To determine an IC50 value for each compound, plot the slope as a function of test compound concentration.

Complex II Activity (%) = (Rate of Sample wells /Rate of Vehicle Control) x 100