Rev. 1

ScienCell™ Research Laboratories

Cytochrome C Oxidase Assay (COX)

Catalog #8278
100 tests in 96-well plate

Product Description

Cytochrome c oxidase, the last enzyme in the mitochondrial respiratory electron transport chain, converts molecular oxygen to water and helps establish mitochondrial membrane potential. Located in the inner mitochondrial membrane, it separates the matrix from the inter-membrane space. This colorimetric assay measures the decrease in absorbance at 550 nm as ferrocytochrome c is oxidized to ferricytochrome c by cytochrome c oxidase. When the outer mitochondrial membrane is intact, cytochrome c cannot be oxidized by cytochrome c oxidase. The integrity of the outer membrane is assessed by measuring cytochrome c oxidase activity in mitochondria with and without the detergent n-Dodecyl β-D-maltoside, which disrupts the outer membrane [1].

Kit Components

Cat. No.	# of vials	Reagent	Quantity	Storage
8278a	1	Assay Buffer	20 mL	4°C
8278b	1	Cytochrome C	10.8 mg	-20°C
8278c	1	DTT	3.08 mg	-20°C
8278d	1	n-Dodecyl β-D-Maltoside solution (100x)	0.25 mL	-20°C

Reagents and Equipment Supplied by User

1. Plate reader
2. 96-well clear plates
3. Ultrapure water (Cat# 0600)
4. Mitochondria isolation kit (Cat# 8268)

Quality Control

Cytochrome c oxidase activity was measured at different concentrations of mitochondria or cell lysate derived from primary cells. The cytochrome c oxidase activity (OD550nm) is proportional to the amount of mitochondria in the reaction.

Product Use

Cytochrome c oxidase kit is used for the fast and simple measurement of cytochrome c oxidase activity [2-3]. In addition, this kit also allows measure intactness of mitochondrial outer membrane. This product is for research purposes only and not for use in animals, humans, or diagnostic procedures.

Shipping

Dry ice.

References:

1. Stieglerova A, Drahota Z, Ost adal B, Houstek J. (2000). "Optimal conditions for determination of cytochrome c oxidase activity in the rat heart." *Physiol Res*, 49(2): 245-250.
2. Trounce I, Kim Y, Jun A, Wallace D. (1996). "Assessment of mitochondrial oxidative phosphorylation in patient muscle biopsies, lymphoblasts, and transmitochondrial cell lines." In *Methods in enzymology* 264: 484-509.
3. Racay P, Tatarková Z, Drgová A, Kaplan P, Dobrota D. (2009). "Ischemia-reperfusion induces inhibition of mitochondrial protein synthesis and cytochrome c oxidase activity in rat hippocampus." *Physiol Res*, 58(1): 127-138.

Procedure

1. **Mitochondria isolation**: Isolate mitochondria from cultured cells or tissue by using mitochondria isolation kit (Cat #8268).
2. **Cytochrome C:** Weigh and dissolve 2.7 mg of cytochrome c in 1 mL of deionized (DI) water. Alternatively, add 4 mL of DI water to the cytochrome c bottle (Cat #8278b) containing 10.8 mg of cytochrome c to make a 0.22 mM solution.
3. **DTT solution:** Add 200 µL of DI water to the DTT vial (Cat #8278c, 3.08 mg) to make a 0.1 M DTT solution. Aliquot and store at -20 °C. Thaw only before use.
4. **Reduced Cytochrome C**: Add 10 µL of DTT solution (Cat #8278c) per mL of cytochrome c solution, mix, and leave at room temperature for 15 to 20 minutes to make reduced cytochrome c solution. The color of the solution should change from dark orange-red to pale purple-red. To confirm the reduction of cytochrome c, dilute the cytochrome c solution mixed with DTT 5-fold with 1x assay buffer (60 µL of cytochrome c solution in 240 µL of assay buffer (Cat #8278a). Aliquot 200 µL into one well of a 96-well plate and measure the absorbance ratio at 550 nm/560 nm; the absorbance ratio should be between 10 and 20.
   Note: If the ratio is less than 10, the cytochrome c has not been sufficiently reduced, and the enzyme activity will not be valid. In this case, add 5 µL of DTT solution, wait 15 minutes, and measure the ratio again. Aliquot and store the remaining reduced cytochrome c at -20°C.
5. **Substrate Preparation:** Warm the assay solutions to room temperature before starting the reaction. Dilute the reduced cytochrome c solution 5-fold with 1x assay buffer to make the substrate (e.g., mix 200 µL of cytochrome c solution with 800 µL of assay buffer).

Measurements (96-well plate)

1. Set the plate reader at 550 nm on a kinetic program:
   Duration: 10 minutes
   Interval: 30 seconds
2. Prepare sample reactions according to the reaction scheme (see below).

Substrate	198-X µL
n-Dodecyl β-D-Maltoside solution	2 µL
Mitochondrial protein (0.5~2 µg)	X µL

   Note: If mitochondrial outer membrane integrity needs to be measured replace n-Dodecyl β-D-Maltoside solution with DI water for intact mitochondria samples.
3. Prepare blank by replacing mitochondria with DI water.
4. Mix the solution by shaking the plate.
5. Immediately read and record decrease in OD for 10 minutes.

Calculations

1. Calculate ΔA/min by using of the maximum linear rate. The oxidation of cytochrome c by cytochrome c oxidase is biphasic reaction with a fast initial burst of activity followed by a slower reaction rate. ΔA= change in OD reading.
2. Calculate cytochrome c oxidase activity of the sample.

$$\text{Unit/mg mitochondria} = \frac{\Delta A/\text{min}}{\varepsilon \times \text{mg mitochondria}}$$

ΔA/min = (change in OD reading)/time
ε = 19.6 mM^{-1} cm^{-1} and ε is extinction coefficient of reduced cytochrome c solution at 550 nm
Unit definition: One unit would oxidize 1.0 μmole reduced cytochrome c per minute at pH 7.2 at 25 ºC

Measuring Mitochondrial Outer Membrane Integrity (96-well plate)

The ratio of cytochrome c activity in mitochondria samples with and without n-Dodecyl β-D-maltoside indicates the integrity of the mitochondrial outer membrane.

Note: Freeze/thaw processes may potentially cause rupture of the membrane of mitochondria. Therefore, freshly prepared tissues are recommended, though frozen tissues could still be used for measuring total activity of cytochrome c oxidase.

1. Measure cytochrome c oxidase activity in intact or lysed mitochondria (including 1X n-Dodecyl β-D-maltoside in the reaction).
2. Calculate the maximum linear rate for each sample.
3. Calculate the degree of mitochondrial integrity.

% mitochondria with intact mitochondria outer membrane:

$$\% = \frac{\Delta A/\text{minute(w/ detergent)} - \Delta A/\text{minute (w/o detergent)}}{\Delta A/\text{minute (w/ detergent)}}$$

ΔA= change in OD reading