Cytochrome C Oxidase Assay (COX)

Cat. No. 8278
100Tests in cuvette

Introduction

Cytochrome c oxidase is the last enzyme in the respiratory electron transport chain of mitochondria. Its main function is to convert molecular oxygen to water and aid in establishing mitochondrial membrane potential. Cytochrome c oxidase locates to the inner membrane which separates the mitochondrial matrix from the inter-membrane space. This colorimetric assay is based on observation of the decrease in absorbance at 550 nm of ferrocytochrome c caused by its oxidation to ferricytochrome c by cytochrome c oxidase.

Kit Components

Cat. No.	# of vials	Reagent	Quantity	Storage
8278a	1	Assay Buffer	100mL	4°C
8278b	1	Cytochrome C(4X)	1.25mL	-20°C
8278c	1	DTT solution	0.2mL	-20°C
8278d	1	n-Dodecyl β-D-Maltoside solution (100X)	1mL	-20°C

Product Use

Cytochrome c oxidase kit is used for the fast and simple measurement of cytochrome c oxidase activity[1-2]. In addition, this kit also allows for the detection of intact mitochondrial outer membrane. This product is for research purposes only and not for use in animals, humans, or diagnostic procedures.

Quality Control

Mitochondria cytochrome c oxidase activity was measured on rodent liver tissue and human dermal fibroblasts (ScienCell™ Cat.No.2300) derived mitochondria in serial dilution. The cytochrome c oxidase activity (OD_{550nm}) is proportional to the amount of mitochondria in reaction.

Reagents and Equipment Supplied by User

1. Spectrophotometer
2. 1mL cuvettes
3. Ultrapure water (ScienCell™ Cat. No. 0600)
4. Mitochondria isolation kit (ScienCell™ Cat. No. 8268)

Shipping and Stability

The kit would be shipped on dry ice and the kit is stable for one year when handled properly

Sample and Buffer Preparation

1. Mitochondria isolation: isolate mitochondria from cultured cells or tissue by using mitochondria isolation kit (ScienCell™ Cat.No.8268).
2. Cytochrome c working solution (1X): thaw and dilute cytochrome c stock solution (8728b) in ultra-pure water (1:4).
3. Substrate: add 10µL DTT solution (8278c) per mL cytochrome c working solution (1X), mix and leave it in room temperature for 15~ 20 minutes.
4. Checking the efficiency of reducing cytochrome c: mix 50 µL substrate with 950 µL of assay buffer (8278a), read its OD at 550nm and 560nm. The optimal ratio (550nm/560nm) should be between 10 and 20.

Procedure for Cytochrome C Oxidase Activity Assay (1mL cuvette)

1. Set the spectrophotometer at 550 nm on a kinetic program:
   Duration: 30 seconds
   Interval: 5 seconds
2. Warm the assay solutions to room temperature before starting the reaction. Mix well.
3. Prepare sample reactions according to the reaction scheme (see below)

Assay Buffer	940-X µL
n-Dodecyl β-D-Maltoside solution	10 µL
Mitochondrial protein (0.5~2 µg)	X µL

4. Mix solution in cuvette.

Note: If several samples are required for measuring, n-Dodecyl β-D-Maltoside solution is recommended to be added just before blanking.

5. Blank spectrophotometer with reaction mixture.
6. Add 50 µL substrate solution and mix (covered by parafilm and shake).
7. **Immediately** read and record decrease in OD for 30 seconds.
8. 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.
9. 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

Example: Calculate the cytochrome c oxidase activity of isolated mitochondria from human dermal fibroblasts

2 μg mitochondria protein was used for cytochrome c oxidase activity assay
The following are the absorbance readings:

Time point (second)	Absorbance at 550nm
0	0.3560
5	0.3306
10	0.3365
15	0.3261
20	0.3192
25	0.3173
30	0.3163

Calculate the linear rate during different time frame:

Time Frame	Linear Rate (ΔA/min)
T_{0s} to T_{10s}	0.1175
T_{10s} to T_{20s}	0.1035
T_{20s} to T_{30s}	0.0175

As T_{20s} is the turning point, then the maxium linear rate for human dermal fibroblasts would be

$$\Delta A/min = \frac{0.1175+0.1035}{2} = 0.1105$$

Human dermal fibroblasts cytochrome c oxidase activity would be

$$\text{Unit/mg mitochondria} = \frac{0.1105}{19.6 \times 0.002} = 2.82 \text{ unit/mg mitochondria}$$

Procedure for Measuring Mitochondrial Outer Membrane Integrity (1mL cuvette)

As cytochrome c oxidase (complex IV) locates in the inner membrane of the mitochondria, cytochrome c could not be oxidized by cytochrome c oxidase when the outer membrane is intact. Therefore, the integrity of the mitochondrial outer membrane is assessed by measuring cytochrome c oxidase activity in the presence and absence of the lipid-like nonionic detergent, n-Dodecyl β-D-maltoside, which could stabilize cytochrome c oxidase dimers in solution at low detergent concentrations[3]. The ratio between activity without and with n-Dodecyl β-D-maltoside presence is a measurement of the integrity of the mitochondrial outer membrane.
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. Approximately 0.5 to 2µg mitochondria suspension is mixed with either assay buffer (measuring cytochrome c oxidase activity in intact mitochondria) or with the assay buffer containing 1X n-Dodecyl β-D-maltoside (measuring total cytochrome c oxidase activity).
2. Blank spectrophotometer with reaction mixture.
3. Add 50 µL reduced cytochrome c and mix (covered by parafilm).
4. **Immediately** read and record increase in OD $_{550nm}$ for 30 seconds.
5. Calculate the maximum linear rate for each sample.
7. 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

References:

1. Trounce IA, Kim YL, Jun AS, Wallace DC. Assessment of mitochondrial oxidative phosphorylation in patient muscle. biopsies, lymphoblasts, and transmitochondrial cell lines. 1996. Methods Enzymol 264: 484–509.
2. Racay P, Tatarkova Z, Drgova A, Kaplan P, Dobrota D. Ischemia-reperfusion induces inhibition of mitochondrial protein synthesis and cytochrome c oxidase activity in rat hippocampus. Physiol Res 58: 127138, 2009.
3. Stieglerova A, Drahota Z, Ostadal B and Houstek J. Optimal conditions for Determination of cytochrome c oxidase activity in the rat heart. 1999. Physiological Research 49: 245-250.