Tribioscience

Cytochrome C Oxidase Assay Kit (100 tests) Catalog Number **TBS2115**

Description

Cytochrome c oxidase [EC 1.9.3.1.] or Complex IV is the fourth complex of the electron Transport Chain located in the mitochondrial, and the principle terminal oxidase of high-affinity oxygen in the aerobic metabolism of all animals, plants, yeasts, and some bacteria. This enzyme is probably unique in providing energy for the cell by coupling electron transport through the cytochrome chain with the process of oxidative phosphorylation.

The colorimetric assay in this kit is based on the observation of the decrease in absorbance at 550 nm of ferrocytochrome c caused by its oxidation to ferricytochrome c by cytochrome c oxidase. It is suitable for the detection of mitochondrial outer membrane integrity and for the detection of mitochondria in subcellular fractions.

Components and Storage

This kit is sufficient for 100 tests. Storage at –20°C

Assay Buffer 5 x	5 mL
Enzyme Dilution Buffer 2x	2 mL
Cytochrome c Solution	1 mL
Dithiothreitol (DTT) Solution	30 µL
n-Dodecyl β-D-maltoside Buffer	2 mL
Cytochrom c Oxidase Positive Control	50 µL

Reagents and Equipment Not Provided

Spectrophotometer
Analytical balance
Ultrapure water (≥18ΩMxcm resistivity at 25°C)

Preparation Instructions

Use ultrapure water for the preparation of reagents.

1x Assay Buffer: Dilute an aliquot of 5x Assay Buffer with ultrapure water. Keep at room temperature.

1x Enzyme Dilution Buffer: Dilute an aliquot of 2x Enzyme Dilution Buffer to 1x working solution with ultrapure water.

0.1 M Dithiothreitol (DTT) Solution: Dilute an aliquot of the 1 M DTT Solution for 10-fold with ultrapure water to a concentration of 0.1 M.

Substrate: add 10µL of the 0.1 M DTT Solution to 2 mL of Cytochrome c solution, mix gently, and wait for 15 minutes at room temperature. The color of the solution should go from dark orange-red to pale purple-red. This is now reduced form of Cytochrome c.

Efficiency of reducing cytochrome c: Mix 10 µL substrate with 190µL of 1x assay buffer for a well. Use 1x Assay buffer to zero the spectrophotometer. Read OD at 550nm/565 mm. The A550/565 ratio should be between 10 and 20.

Procedure

A. Cytochrome c oxidase/Complex IV activity

The absorption of cytochrome c at 550 nm changes with its oxidation state. This property is the basis for the assay. Cytochrome c is reduced with dithiothreitol(DTT) and then re-oxidized by the cytochrome c oxidase. The difference in extinction coefficients ($\Delta\varepsilon^{mM}$) between reduced and oxidized cytochrome c is 21.84 at 550 nm.

The oxidation of cytochrome c by cytochrome c oxidase is a biphasic reaction with a fast initial burst of activity initial reaction rate is measured during the first 45 seconds of the reaction.

Total volume of the reaction is 220 µL (see Table 1).

Table 1. Reaction Scheme

Sample	Assay Buffer (µL)	Enzyme Buffer (µL)	Sample (µL)	Substrate Solution (µL)
Blank	190	20	–	10
Unknown sample	190	(20–x)	x	10

Spectrophotometer settings:

Follow the decrease in absorption at 550 nm at room temperature (25°C) using a kinetic program: 5 second delay; 10 second interval; 6 readings. Set up the instrument prior to starting any reaction.

1. Add 190µL of 1x Assay Buffer to a well in 96-well plate and zero the spectrophotometer.
2. Add a suitable volume of enzyme solution or mitochondrial suspension to the well, and bring the reaction volume to 210µL with 1x Enzyme Dilution Buffer. Mix by pipetting up and down.
3. Start the reaction by adding 10µL of Substrate Solution. Mix by pipetting up and down.
4. Read the A_{550}/minute **immediately** due to the rapid reaction rate of this enzyme.
5. Background values are expected to be between 0.001 and 0.005 A_{550}/minute.
6. Calculate the activity of the sample.

Units/m l= (A/minx Dil x 0.22) / (Sample Vol x21.84)

A/min = $A/minute_{(sample)}$ – $A/minute_{(blank)}$
Dil = dilution factor of sample
0.22 = reaction volume in ml
Sample vol = volume of sample or enzyme in µl
21.84 = $\Delta\varepsilon^{mM}$ between ferrocytochrome c and

ferricytochrome c at 550 nm

Unit definition: One unit will oxidize 1.0 μmole of ferrocytochrome c per minute at pH 7.0 at 25°C.

B. Measurement of the outer membrane integrity of mitochondria

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 detergent, *n*-dodecyl β-D-maltoside, which would stabilize cytochrome c oxidase dimer in solution at low detergent concentrations. The ratio between activity with and without n-dodecyl β-D-maltoside present is a measurement of the integrity of the mitochondrial outer membrane.

Use of frozen tissues may cause rupture of the subcellular organelles and therefore, it is recommended to use freshly prepared tissues. Percent damage to outer mitochondrial membranes from various tissues.

1. Dilute two parallel samples of the mitochondrial suspension to 0.1–0.2 mg protein/ml with either 1xEnzyme Dilution Buffer (cytochrome c oxidase activity in **intact** mitochondria) or with *n*-Dodecyl β-D-maltoside Dilution Buffer (**total** cytochrome c oxidase activity).

2. Incubate the samples at 2–8°C for at least for 10 minutes before assaying.

3. Take 0.1–0.5 µg of mitochondrial protein and assay for cytochrome c oxidase activity (Section A, steps 1-6).

4. Determine the ΔA550/min for each sample:

$$A_{(intact)} = A_{(intact\ sample)} - A_{(blank)}$$
$$A_{(total)} = A_{(total\ sample)} - A_{(blank)}$$

5. Calculate the degree of mitochondrial integrity:
% mitochondria with undamaged outer membranes

$$\% = [\ (A_{(total)} - A_{(intact)}) \times 100] / A_{(total)}$$

Related Product

Protein Assay Kit (TBS2005)
Cytochrome C Reductase Activity Assay (TBS2116)
Non-esterified Fatty Acid Assay (TBS2203)
Glycerol Colorimetric / Fluorometric Assay (TBS2204)
BrdU Cell Proliferation Colorimetric Assay (TBS2086)

References

1. Storrie, B., and Madden, E.A., Methods Enzymol., 182, 214-215 (1990).
2. Hovius, R., et al., Biochim. Biophys. Acta, 1021, 217-226 (1990).
3. Rasmussen, U.F., and Rasmussen, H.N., Mol. Cell. Biochem., 208, 37-44 (2000).
4. Musatov, A., *et al*., Biochem., 39, 12996-13003 (2000)

Storage/Stability

The kit ships on wet ice and storage at –20°C is recommended. When stored unopened, the components in this kit are stable for 24 months. After the initial thawing of the 1 M Dithiothreitol Solution, divide the solution into undiluted working aliquots (still at 1 M concentration) and store at -20°C.

Precautions and Disclaimer

This kit is for R&D use only, not for drug, household, or other uses.