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ab239711 Cytochrome C Oxidase Assay Kit

For the measurement of Cytochrome C Oxidase activity in isolated mitochondria and mitochondria-containing tissue extracts.

This product is for research use only and is not intended for diagnostic use.

Table of Contents

1. **Overview**

Cytochrome C Oxidase Activity Assay Kit (ab239711) is simple, fast and high-throughput adaptable. This assay kit can be used for purified mitochondria or tissue extracts containing mitochondria. The kit provides for the fast and simple measurement of Cytochrome C oxidase activity on a 96-Well Clear Plate. The activity of the enzyme is determined colorimetrically by following the oxidation of reduced Cytochrome C as an absorbance decrease at 550 nm. The overall reaction is as follows:

$$4\ \text{Cyt c}^{-} + 4\text{H}^{+} + 4\text{H}^{+}\ (\text{IN}) + \text{O}_2 \longrightarrow 4\ \text{Cyt c}^{+} + 2\text{H}_2\text{O} + 4\text{H}^{+}\ (\text{OUT})$$

2. **Protocol Summary**

Prepare test samples (isolated mitochondria or cell/tissue extracts) using an appropriate method.

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Add samples or blank (Dilution Buffer I) to wells.

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Add reduced Cytochrome C to all wells.

⇩

Immediately read the reduction in OD 550nm over a period of 30-45 min.

3. **General guidelines, precautions, and troubleshooting**

- Please observe safe laboratory practice and consult the safety datasheet.
- For general guidelines, precautions, limitations on the use of our assay kits and general assay troubleshooting tips, particularly for first time users, please consult our guide: www.abcam.com/assaykitguidelines
- For typical data produced using the assay, please see the assay kit datasheet on our website.

4. Materials Supplied and Storage

- Store kit at -20°C in the dark immediately on receipt and check below for storage for individual components.
- Aliquot components in working volumes before storing at the recommended temperature.
- Avoid repeated freeze-thaws of reagents.

Item	Quantity	Storage temperature (before prep)	Storage temperature (after prep)
Cytochrome Oxidase Assay Buffer	25 mL	-20°C	N/A
Dilution Buffer I	10 mL	-20°C	N/A
DTT IV	1 mL	-20°C	N/A
Cytochrome C	2 vials	-20°C	-20°C
96-Well Clear Plate	1 unit	-20°C	N/A

5. Materials Required, Not Supplied

These materials are not included in the kit, but will be required to successfully perform this assay:

- Multi-well spectrophotometer capable of reading absorbance.

6. Reagent Preparation

- Before using the kit, spin tubes and bring down all components to the bottom of tubes.
- Prepare only as much reagent as is needed on the day of the experiment.

6.1 Cytochrome Oxidase Assay Buffer

Ready to use as supplied. Warm to room temperature prior to use.

6.2 Dilution Buffer I

Ready to use as supplied. Keep on ice throughout assay.

6.3 DTT IV

Ready to use as supplied. Aliquot and store at -20°C. Thaw just before use.

6.4 Cytochrome C

- Reconstitute each vial with 1 mL of Cytochrome Oxidase Assay Buffer. Mix thoroughly by vortexing.
- Add 5 µL of DTT IV solution. Mix well and wait 15 min at room temperature. Keep this working solution at room temperature.
- After the assay is completed, aliquot and save the rest of the Cytochrome C solution at -20°C. This is now the reduced form of Cytochrome C.

7. Sample Preparation

General sample information:

- We recommend performing several dilutions of your sample to ensure the readings are within the standard value range.
- We recommend that you use fresh samples for the most reproducible assay.
- Cell/tissue lysates should be freshly prepared and assayed immediately after homogenization. Storage of lysate is not recommended.
- Samples should be kept on ice at all times during processing including centrifugation steps.

7.1 Isolate mitochondria from cultured cells, yeast or tissues by using ab65320 (Mitochondria/Cytosol Fractionation Kit) or ab178779 (Mitochondrial Yeast Isolation Kit) or use cell or tissue lysate (Prepared by cell lysis buffer containing non-ionic and non-denaturing detergents, for example 1% Triton X in PBS).

7.2 The recommended range of purified mitochondria is 0.5- 5 µg and tissue extract is 1-60 µg per reaction. Dilute the test samples, if needed, with Dilution Buffer I.

8. **Assay Procedure**

– Assay all samples in duplicate.

8.1 Efficiency of reduction of Cytochrome C

8.1.1 In a 96-Well Clear Plate, mix 20 µL of reduced Cytochrome C with 100 µL of Cytochrome Oxidase Assay Buffer.

8.1.2 Prepare a parallel well as blank with only Cytochrome Oxidase Assay Buffer.

8.1.3 Read OD at 550 nm. The OD at 550 nm of reduced Cytochrome C is between 0.2-0.6. If not, add 5 µl of DTT/mL of reconstituted Cytochrome C and wait for 15 min to read the OD again.

8.2 Cytochrome C/Cytochrome c preparation:

8.2.1 Prepare a 1:6 dilution of reduced Cytochrome C by using pre-warmed Cytochrome Oxidase Assay Buffer (one part of Cytochrome C to 5 parts of buffer) in a separate tube depending on the number of assay samples and controls.

8.2.2 Prepare 120 µL of diluted Cytochrome C per reaction.

8.3 Complex IV activity assay:

8.3.1 Before the reaction, set the spectrophotometer at 550 nm on kinetic program for 30-45 min at 30 second interval.

8.3.2 Add the test samples (approx. volume 5-10 µL) to each well of a 96-Well Clear Plate. For negative control (Blank), add equal volume of Dilution Buffer I.

8.3.3 Add 120 µL of the diluted reduced Cytochrome C from Step 8.2 to each sample and control using a multichannel pipette. Shake and immediately read and record the decrease in OD over a period of 30-45 min.

ΔNote: The rate of the reaction is relative to a control or normal sample. The rate is calculated in the linear range.

9. **Data Analysis**

9.1 Calculate rate of the reaction by calculating change in OD: ΔOD/min by using the maximum linear rate.

9.2 The oxidation of Cytochrome C by complex IV is a biphasic reaction with an initial fast burst followed by slower activity. The rate of the reaction will be calculated in the linear range.

9.3 Average the duplicate reading for each standard, control and sample.

$$Cytochrome\ c\ oxidase\ activity\ (units/protein) = \frac{\frac{\Delta OD}{\Delta t}}{E * protein\ (mg)}$$

Where:

ΔOD = difference in OD between time t1 and t2 (see Figure 1).

Δt = difference in time (min) between t1 and t2 (see Figure 1).

E = molar extinction coefficient of reduced Cytochrome C at 550 nm in the given 96-Well Clear Plate (7.04 $mM^{-1}cm^{-1}$).

Protein = the amount of protein used per reaction well (mg).

Unit definition: 1 unit would oxidize 1 μmol of reduced Cytochrome C per min at 25°C and pH 7.2.

10. **Typical Data**

Data provided **for demonstration purposes only**.

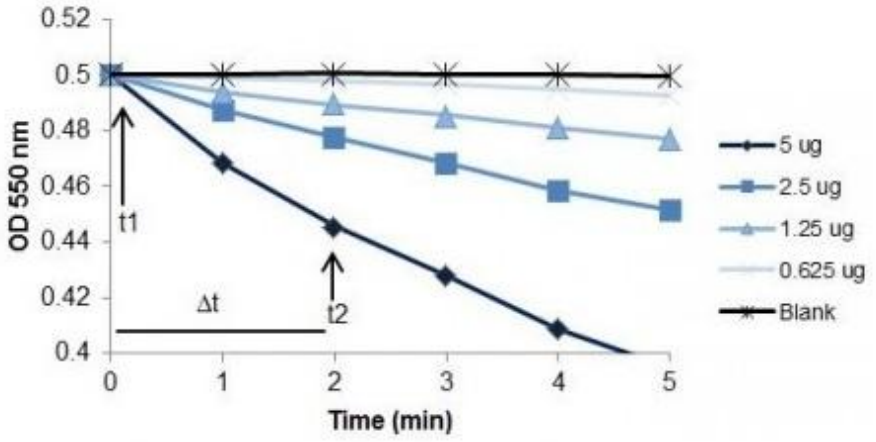

Figure 1. Cytochrome Oxidase Activity: Purified mitochondria were used to calculate a decrease in OD at 550 nm (0.625-5 µg/reaction). In Blank, no change in the OD was observed. Rate is calculated by subtracting the initial OD reading from the final OD, t1 and t2 represents linear rate of reaction.

11. **Notes**

Technical Support

For all technical or commercial enquiries please go to:
https://www.abcam.com/en-us/contact-us
https://www.abcam.cn/contact-us (China)
https://www.abcam.co.jp/contact-us (Japan)