Product Information

Cytochrome Oxidase Activity Colorimetric Assay Kit

I. Kit Contents:

Components	K2020-100 100 assays	Cap Color	Part Number
Cytochrome Oxidase Assay Buffer	25 ml	WM	K2020-C-1
Enzyme Dilution Buffer	8 ml	NM	K2020-C-2
1 M DTT	1 ml	Green	B7294
Cytochrome c	2 vials	Red	K2020-C-3
96-well Plate	1 Plate	---	K2020-C-4

Add 150 µl of DMSO, and mix well before use.

II. Introduction:

The cytochrome c oxidase (complex IV) is a transmembrane protein complex exists in bacteria and the mitochondrion of eukaryotes. Cytochrome c Oxidase is the last enzyme in the respiratory electron transport chain located in the bacterial or mitochondrial membrane. Cytochrome c Oxidase receives an electron from each of four cytochrome c molecules, and transfers it to O_2, converting O_2 to two molecules of H_2O. In the process, it binds four protons from the inner aqueous phase to make H_2O, and translocates four protons across the membrane to establish a proton electrochemical potential that is used to synthesize ATP by the ATP synthase. Cytochrome c Oxidase provides energy to the cell by coupling electron transport through the respiratory electron transport chain with oxidative phosphorylation.

Cytochrome Oxidase Activity Colorimetric Assay Kit is fast, simple and high-throughput adaptable. This assay kit can be used for cells/tissue extracts containing mitochondria or purified mitochondria. The activity of cytochrome oxidase is detected colorimetrically by following the oxidation of decreased Cytochrome c as an absorbance decrease at 550 nm.

III. Storage and Handling:

Store kit at -20℃, protected from light. Warm Assay Buffer to room temperature before use. Keep Enzyme Dilution Buffer on ice. Read the entire protocol before performing the assay.

IV. Reagent Preparation and Storage Conditions:

DTT: Aliquot and store at -20℃. Thaw just before use.

Cytochrome c: Reconstitute each vial with 1 ml of Cytochrome Oxidase Assay Buffer. Mix by vortexing to dissolve completely. Add 5 µl of DTT solution. Mix well and wait for 15 min. at room temperature. Keep this working solution at room temperature. After assay is completed, aliquot and save rest of the Cytochrome c solution at -20℃. This is now reduced form of Cytochrome c.

V. Complex IV Activity Assay Protocol:

1. Efficiency of Reduction of Cytochrome c: In a 96-well plate, mix 20 µl of reduced Cytochrome c with 100 µl of Cytochrome Oxidase Assay Buffer. Prepare a parallel well as blank with only Assay Buffer. 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 again the OD.

2. Sample Preparation: Isolate mitochondria from cultured cells, yeast or tissues by using Mitochondria/Cytosol Fractionation Kit or Yeast Mitochondria Isolation Kit or use cell or tissue lysate. 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 by Enzyme Dilution Buffer.

3. Cytochrome c preparation: Prepare 1:6 dilution of 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. Prepare 120 µl of diluted Cytochrome c per reaction.

4. Complex IV Activity Assay: Before the reaction, set the spectrophotometer at 550 nm on kinetic program for 30 - 45 minutes at 30 sec interval. Add the test samples (approx. volume 5 - 10 µl) to each well of a 96-well plate. For negative control (Blank), add equal volume of Enzyme Dilution Buffer. Add 120 µl of the diluted Cytochrome c from Step 3 to each sample and control using a multichannel pipette. Shake and immediately read and record decrease in OD over a period of 30 - 45 min.

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

5. Calculations: Calculate rate of the reaction by calculating change in OD: ΔOD/min by using the maximum linear rate. The oxidation of Cytochrome c by complex IV is biphasic reaction with an initial fast burst followed by slower activity. The rate of the reaction will be calculated in the linear range.

Cytochrome Oxidase Activity (Units/mg) = (ΔOD /Time)/(Ɛ × protein)

Where: ΔOD is the difference in OD at time (t1) and time (t2)

Δt is the difference in time (min); t1 - t2.

Ɛ is the molar extinction coefficient of reduced Cytochrome c at 550 nm in the given 96-well plate; 7.04 mM-1cm-1

Protein is the conc. of sample (mg) used per reaction.

Unit definition: One unit would oxidize 1 µmole reduced Cytochrome c per minute at pH 7.2 at 25℃.

Frequently Asked Questions:

1. Can this kit be used with samples like bacteria, plants, drosophila, yeast etc?

We have optimized the kit with mammalian samples. However, theoretically these kits should work with samples from multiple species/sources. Since the optimal conditions depend on the sample type, the protocol has to be be adapted to fit the samples for efficient results. Please refer to this kits citations to see what kind of samples have been used with this kit other than mammalian samples.

2. Can we use frozen samples with this assay?

Fresh samples are always preferred over frozen samples. However, frozen samples can also be used, provided, they were frozen right after isolation, were not freeze thawed multiple time (for which we recommend aliquoting the samples before freezing) and have been frozen for relatively short periods.

3. Can we use a different wavelength than recommended for the final analysis?

It is always recommended to use the exact recommended wavelength for the most efficient results. However, most plate readers have flexibility in their band width of detection in increments of +/- 10 nm. Depending on this flexibility range, you can deviate from the recommended wavelengths within limits.

4. What is the exact volume of sample required for this assay?

There is no specific volume we can recommend for the amount any sample to be used since it is completely sample concentration and quality based. You have to do a pilot expt with multiple sample volumes to determine the optimal volume which gives a reading within the linear range of the standard curve. Please refer to the citations for this product to see what other clients have used with similar sample types.

5. What is the shelf life of this kit?

This kit is good for 12 months from the date of shipment in the unopened form when stored at the appropriate temperature and appropriate conditions. After opening and reconstitution, some of the components in this kit are good for 2 months at -20℃. Please refer to the datasheet for storage information and shelf life of each of the components.

6. Why are my standard curve values lower than those shown on the datasheet?

There are multiple factors which influence the signals like the incubation times, room temperature, handling etc. In general, to increase the value of the standards, you can increase the incubation time. As long as the standard curve is linear, it should be fine to use, since all of your samples will also be measured under the same conditions on this curve.

8. How do I normalize my samples against protein concentration

You can use a protein quantitation assay on the supernatants you get from cell/tissue lysates or with any other liquid sample in the assay buffer.

9. Can we use an alternate buffer for sample preparation (cell lysis, sample dilutions etc)?

Our assay buffers are optimized for the reactions they are designed for. They not only contain some detergents for efficient lysis of your cells/tissue, but also contain some proprietary components required for the further reactions. Therefore, we highly recommend using the buffers provided in the kit for the best results.

10. Should I make a standard curve for every expt I do, or is one curve/kit enough?

Yes, It is recommended to do the standards every time you do the expt. There is always a chance that something was done differently that day and we do not want any conditions to differ between standards and samples.

For research use only! Not to be used in humans.

Our promise

If the product does not perform as described on this datasheet, we will offer a refund or replacement. For more details, please visit http://www.apexbt.com/ or contact our technical team.

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