ab109911
Complex IV Rodent Enzyme Activity Microplate Assay Kit

Instructions for use:

To determine the activity of Cytochrome C Oxidase in Mouse and Rat samples

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

Version 4 Last Updated 21 January 2019

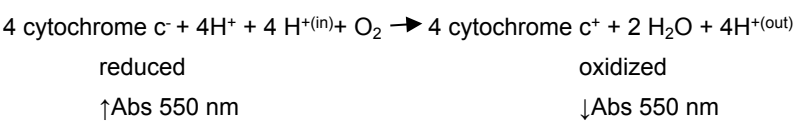
Table of Contents

1. BACKGROUND

Abcam's enzyme activity assays apply a novel approach, whereby target enzymes are first immunocaptured from tissue or cell samples before subsequent functional analysis. All of our ELISA kits utilize highly validated monoclonal antibodies and proprietary buffers, which are able to capture even very large enzyme complexes in their fully-intact, functionally-active states.

Capture antibodies are pre-coated in the wells of premium Nunc MaxiSorp™ modular microplates, which can be broken into 8-well strips. After the target has been immobilized in the well, substrate is added, and enzyme activity is analyzed by measuring the change in absorbance of either the substrate or the product of the reaction (depending upon which enzyme is being analyzed). By analyzing the enzyme's activity in an isolated context, outside of the cell and free from any other variables, an accurate measurement of the enzyme's functional state can be understood.

ab109911 The Complex IV Rodent Enzyme Activity Microplate Assay Kit (MS444) is used to determine the activity of the cytochrome c oxidase enzyme (EC 1.9.3.1) in a mouse sample. Complex IV is immunocaptured within the wells and activity is determined colorimetrical by following the oxidation of reduced cytochrome c as an absorbance decrease at 550 nm. The overall reaction is as follows:



ab109911 has been developed for use with mouse samples. Separate kits are available for human samples.

INTRODUCTION

This assay is designed for use with purified mitochondria. However, homogenized tissue and whole cells can also be used. Samples should be solubilized, the protein extracted and measured within the linear range as described below. A control or normal sample should always be included in the assay as a reference. Also include a null or buffer control to act as a background reference measurement.

Typical linear ranges:

Tissue extracts	10-100 µg / 200 µl
Tissue mitochondria	10-100 µg / 200 µl

2. ASSAY SUMMARY

Prepare Sample (1-3 hours)

- Homogenize sample, pellet, and adjust to 5.5 mg/ml in Solution 1.
- Perform detergent extraction with 1/10 volume detergent followed by 16,000 rpm centrifugation for 20 minutes. Take supernatant.
- Adjust concentration to recommended dilution for plate loading.

Load Plate (3 hours)

- Load sample(s) on plate being sure to include positive control sample and buffer control as null reference.
- Incubate 3 hours at room temperature.

Measure (2 hours)

- Rinse wells twice with Solution 1.
- Prepare appropriate volume of assay solution and add to wells.
- Measure OD550 at 1-5 minute intervals for 2 hours at 30°C.

3. PRECAUTIONS

Please read these instructions carefully prior to beginning the assay.

- All kit components have been formulated and quality control tested to function successfully as a kit.
- We understand that, occasionally, experimental protocols might need to be modified to meet unique experimental circumstances. However, we cannot guarantee the performance of the product outside the conditions detailed in this protocol booklet.
- Reagents should be treated as possible mutagens and should be handle with care and disposed of properly. Please review the Safety Datasheet (SDS) provided with the product for information on the specific components.
- Observe good laboratory practices. Gloves, lab coat, and protective eyewear should always be worn. Never pipet by mouth. Do not eat, drink or smoke in the laboratory areas.
- All biological materials should be treated as potentially hazardous and handled as such. They should be disposed of in accordance with established safety procedures.

4. STORAGE AND STABILITY

Store kit in the dark immediately upon receipt. Kit has a storage time of 1 year from receipt, providing components have not been reconstituted.

Refer to list of materials supplied for storage conditions of individual components. Observe the storage conditions for individual prepared components in the Materials Supplied section.

Aliquot components in working volumes before storing at the recommended temperature.

5. LIMITATIONS

- Assay kit intended for research use only. Not for use in diagnostic procedures.
- Do not mix or substitute reagents or materials from other kit lots or vendors. Kits are QC tested as a set of components and performance cannot be guaranteed if utilized separately or substituted.

6. MATERIALS SUPPLIED

Item	Amount	Storage Condition (Before Preparation)
Tube 1 (Buffer)	10 mL	4°C
Detergent	1 mL	4°C
Reagent C (Reduced Cytochrome c)	1 mL	-80°C
96-well microplate (12 strips)	1	4°C

7. MATERIALS REQUIRED, NOT SUPPLIED

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

- Microplate reader capable of measuring absorbance at OD=550nm
- Ultrapure water or other type of double distilled water (ddH_2O)
- Pipettes and pipette tips, including multi-channel pipette
- Assorted glassware for the preparation of reagents and buffer solutions
- Tubes for the preparation of reagents and buffer solutions
- Dounce homogenizer (if using tissue)
- Protein assay method

8. TECHNICAL HINTS

- **This kit is sold based on number of tests. A 'test' simply refers to a single assay well. The number of wells that contain sample, control or standard will vary by product. Review the protocol completely to confirm this kit meets your requirements. Please contact our Technical Support staff with any questions.**
- Selected components in this kit are supplied in surplus amount to account for additional dilutions, evaporation, or instrumentation settings where higher volumes are required. They should be disposed of in accordance with established safety procedures.
- Avoid foaming or bubbles when mixing or reconstituting components.
- Avoid cross contamination of samples or reagents by changing tips between sample, standard and reagent additions.
- Ensure plates are properly sealed or covered during incubation steps.
- Ensure all reagents and solutions are at the appropriate temperature before starting the assay.
- Samples which generate values that are greater than the most concentrated standard should be further diluted in the appropriate sample dilution buffer.
- Make sure you have the right type of plate for your detection method of choice.
- Make sure all necessary equipment is switched on and set at the appropriate temperature.

9. SAMPLE PREPARATION

General Sample Information

- We recommend that you use fresh samples. If you cannot perform the assay at the same time, we suggest that you complete the Sample Preparation step before storing the samples. Alternatively, if that is not possible, we suggest that you snap freeze samples in liquid nitrogen upon extraction and store the samples immediately at -80°C. When you are ready to test your samples, thaw them on ice. Be aware however that this might affect the stability of your samples and the readings can be lower than expected.

9.1. Prepare the buffer solution by adding Tube 1 (10 mL to 190 mL deionized H_2O. Label this solution as Solution 1.

9.2. Pellet the sample by centrifugation and estimate the volume of the pellet or homogenate

9.3. Resuspend the sample by adding 5 volumes of Solution 1, e.g. if the pellet appears to have a volume of 20 µL add 100 µL of Solution 1.

9.4. The sample must be homogenous before detergent extraction. Therefore, resuspend the sample thoroughly by pipetting (cultured cells), or homogenize with a microtissue grinder. Determine the protein concentration by a standard method and then adjust the concentration to 5.5 mg/mL.

NOTE: *The optimal protein concentration for detergent extraction is 5 mg/mL.*

9.5. Add 1/10 volume of Detergent to the sample (e.g. for a sample volume of 90 µL, add 10 µL of Detergent). The sample is now 5 mg/mL.

9.6. Mix immediately and then incubate the sample on ice for 30 minutes.

9.7. Spin in table top microfuge at maximum speed (~16,000 rpm) for 20 minutes at 4°C.

9.8. Carefully collect the supernatant and save as sample. Discard the pellet.

9.9. The microplate wells are optimized for 200 µL sample volume, so dilute samples to the following recommended concentrations by adding Solution 1:

Sample type	Volume
Tissue extracts	50 µg / 200 µL
Tissue mitochondria	25 µg / 200 µL

9.10. Keep diluted samples on ice until ready to proceed to the Assay Method.

10. ASSAY PROCEDURE

- **Equilibrate all materials and prepared reagents to correct temperature prior to use.**
- **We recommended to assay all standards, controls and samples in duplicate.**
- **Prepare all reagents, working standards, and samples as directed in the previous sections.**

10.1. **Plate Loading**

10.1.1. Add 200 µl of each diluted sample into individual wells on the plate. Include a normal sample as a positive control. Include a buffer control (200 µl Solution 1) as a null or background reference.

10.1.2. Incubate the plate for 3 hours at room temperature.

10.2. **Measurement**

10.2.1. The bound monoclonal antibody has immobilized the enzyme in the wells. Empty the wells by quickly turning the plate upside down and shaking out any remaining liquid.

10.2.2. Add 300 µL of Solution 1 to each well.

10.2.3. In a sealable tube prepare an appropriate amount of Assay Solution using Reagent C and Solution 1. Mix gently by inversion. See table below for amounts required. Set Assay Solution aside.

No. of Strips	REAGENT C (µl)	SOLUTION 1 (ml)
1	84	1.67
2	167	3.33
3	250	5.00
4	333	6.67
5	417	8.33
6	500	10.00
7	583	11.67
8	667	13.33
9	750	15.00
10	833	16.67
11	917	18.33
12	1000	20.00

10.2.4. Set up the plate reader to a kinetic program to measure absorbance at 550 nm at 30°C for 120 minutes, with measurement interval of approximately 1 minute (however a longer measurement interval may be used if necessary).

10.2.5. Empty wells and add 300 µl Solution 1. Repeat this wash step.

10.2.6. Empty the wells and add 200 µl of Assay Solution to each well carefully to avoid the formation of bubbles. Any bubbles should be popped with a fine needle as rapidly as possible.

10.2.7. Set plate in plate reader and begin recording immediately.

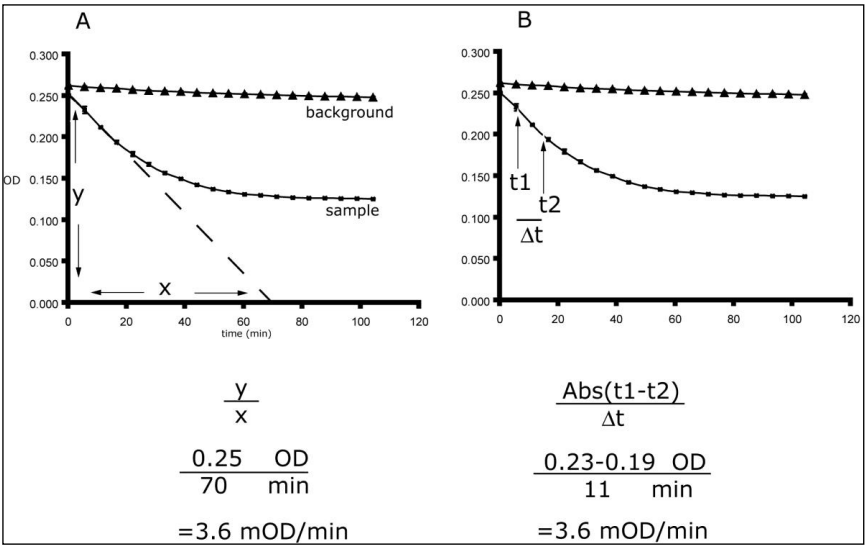
11. CALCULATIONS

Calculation of Complex IV activity

Since the Complex IV reaction is product inhibited, the rate of activity is always expressed as the initial rate of oxidation of cytochrome c. This oxidation is seen as a decrease in absorbance at 550 nm. The initial rate should be a linear decrease. At lower activity levels the linear range is extended.

To determine the activity in the sample, calculate the slope by using microplate software or by manual calculations using one of the two methods shown below. Compare the sample rate with the rate of the control (normal) sample and with the rate of the null (background) to get the relative Complex IV activity.

$$\text{Rate (OD/min)} = \frac{\text{Absorbance 1} - \text{Absorbance 2}}{\text{Time (minutes)}}$$



A: The rate is determined by calculating the gradient of the initial slope over the linear region.

B: The rate is determined by calculating the slope between two points within the linear region.

12. DATA

Reproducibility

Typical intra-assay variations (same day, same sample) <10%

Typical inter-assay variation (day to day, same sample) <10%

Reactivity

Species Reactivity: Mouse, Rat

Separate kits are available for human samples.

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