Version 1 updated 26 February 2020

ab109878 Complex IV Rodent Enzyme Activity Dipstick Assay Kit

View Complex IV Rodent datasheet:

www.abcam.com/ab109878

(use www.abcam.cn/ab109878 for China, or www.abcam.co.jp/ab109878 for Japan)

For the measurement of Complex IV activity in mouse and rat samples

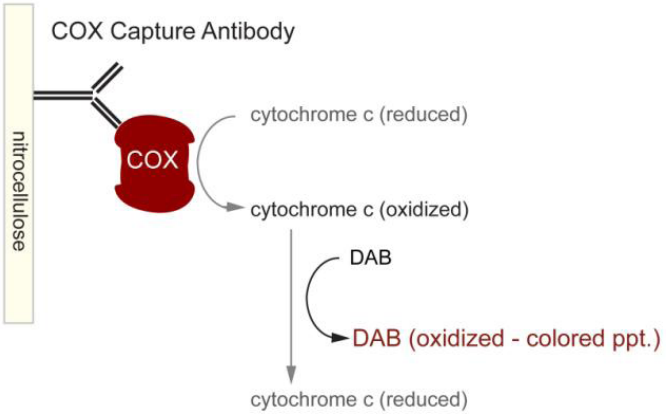
This product is for research use only and is not intended for diagnostic use.

Table of Contents

1. Introduction

ab109878 is used to quantify the activity of the Complex IV (COX) enzyme complex (EC 1.9.3.1) from rat and mouse samples; isolation of mitochondria is not necessary. Using an antibody immobilized on the dipstick that specifically recognizes COX, the enzyme complex is immno-captured (i.e immuno-precipitated in active form) and enzyme activity is determined directly on the dipstick. Using a traditional method for determining COX enzyme activity by histo-chemical methods and in-gel activity assays, Di-amino benzidinetetrachloride (DAB) serves as the reporter of COX activity (Figure 1A). The greater the signal of precipitated DAB, the greater the amount of COX enzyme complex (Figure 1B). In addition, the reaction is cyanide sensitive. The signal intensity is best measured by a dipstick reader or may be analyzed by another imaging system.

(A)



(B)

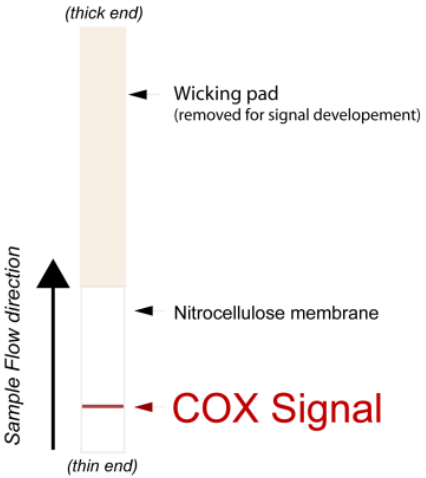


Figure 1. Schematics of the COX Activity reaction and a fully developed COX activity dipstick. (A) Mechanism of the COX activity assay; the immunocaptured COX dipstick is immersed in a solution containing reduced cytochrome c and DAB. COX then generates oxidized cytochrome c, which in turn oxidizes DAB to form a red-colored precipitate at the antibody line. (B) A COX activity dipstick fully developed (dipstick wick is removed before color development). The anti-COX mAb is stripped ~7 mm from the bottom of the dipstick.

2. Quick Assay Procedure

Δ Note: this procedure is provided as a quick reference for experienced users. Follow the detailed procedure when performing the assay for the first time.

Prepare samples.

⇩

Bring sample volume up to 25 µl by adding Buffer A.

⇩

Add sample and 25 µl of Buffer B to a microplate well.

⇩

Add one dipstick to each sample microplate well.

⇩

Allow entire contents to wick up onto the dipstick (15 - 45 minutes).

⇩

Add 30 µl of Buffer C to each microplate well containing a dipstick.

⇩

Allow microplate well contents to wick for 10 minutes.

⇩

Prepare Activity Buffer.

⇩

Add 300 µl of Activity Buffer for each sample into an empty microplate well.

⇩

Remove wicking pad and transfer each dipstick to microplate wells containing Activity Buffer and incubate for 45 to 60 minutes.

⇩

Add dipsticks to 300 µl of deionized water. Wash for 10 minutes.

⇩

Dry dipsticks and measure signal

3. Materials Supplied and Storage

Store dipsticks at room temperature in their provided container and out of direct sunlight. High humidity conditions should be avoided. For long term storage, store Buffer A, B, and C at -20°C.

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

Avoid repeated freeze-thaws of reagents.

Item	Quantity (90 ct)	Quantity (30 ct)	Storage temperature
Dipsticks	90 units	30 units	RT
Buffer A (Extraction buffer)	3 x 15 mL	15 mL	4°C
Buffer B (Blocking buffer)	3 x 2 mL	2 mL	4°C
Buffer C (Wash buffer)	3 x 2 mL	2 mL	4°C
Tube 1 (DAB 100X stock)	3 x 100 µL	100 µL	-80°C
Reduced Cytochrome C	3 x 500 µL	500 µL	-80°C
Tube 3 (Buffer for activity assay)	3 x 10 mL	10 mL	4°C
96-Well Costar Plate	6 units	2 units	RT

4. Materials Required, Not Supplied

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

- Dipstick reader or other imaging system
- Method for determining protein concentration
- Pipetting devices
- Protease inhibitors (Protease inhibitors should be added during the extraction procedure. We recommend any off the shelf cocktail of protease inhibitor)

5. Sample Preparation

Sample preparation is a critical step in this procedure. The effective solubilization of the mitochondrial membranes in the sample is necessary. Please choose the sample preparation method that best fits your sample type. Follow each of the protocol steps carefully.

Note: Samples must be kept on ice.

5.1 Tissue Sample

1. Begin with approximately 25 mg of sample
2. Add 5 volumes/ weight of prechilled Buffer A to the sample (e.g. if the total sample weight is 50 mg, add 250 µl of Buffer A).
3. Homogenize the sample.
4. Keep the sample on ice for 20 minutes and mix intermittently.
5. Spin the cell extract in a microcentrifuge at 16,000 g for 20 minutes at 4°C.
6. Collect the supernatant and determine the protein concentration of the sample extract. Protein concentration should be approximately 1 mg/ml.
7. Proceed directly to Dipstick Procedure or make aliquots and freeze samples at -80°C.

5.2 Cell Culture Sample

1. Add 5 volumes/ cell pellet volume of prechilled Buffer A to a cell pellet (e.g. if the cell pellet is 50 µl in volume, add 250 µl of Buffer A).
2. Keep on ice for 20 minutes, mixing intermittently.
3. Spin the cell extract in a micro-centrifuge at 16,000 g for 20 minutes at 4°C.
4. Collect the supernatant and determine the protein concentration of the sample extract. Protein concentration should be ≥ 1 mg/mL.
5. Proceed directly to Dipstick Procedure or make aliquots and freeze samples at -80°C.

6. Dipstick Assay Procedure

The assay is most accurate with a user established standard curve for interpolation of the signal intensity. Following the protein concentration ranges as defined in Table 1, generate a standard curve using a positive control sample.

Sample Type	Working Range
Mouse Tissue	5 - 75 µg (200-3000 µg/mL)
Rat Tissue	5 - 75 µg (200-3000 µg/mL)

Table 1. Suggested working range for different sample types

1. Load the amount of protein that corresponds toward the high end of the user generated standard curve (~3/4 of the high end).

2. Dilute samples to desired concentration with Buffer A.

3. Add 25 µl of diluted Sample and 25 µl of Buffer B to a microplate well and mix.
   Note: If protein concentration is too low, 100 µl reaction volumes are possible. Be sure to keep the ratio of diluted sample to Buffer B constant.
   Gently add a dipstick to the microplate well (place the thin/nitrocellulose end of dipstick down).

4. Allow the sample to wick up into the dipstick. (This step takes 15 - 45 minutes depending on sample viscosity and total volume).
   Note: The entire sample volume has to be absorbed by the dipstick before proceeding to the next step, but do not allow the dipstick to dry at any time during this procedure.

5. Wash by adding 30 µl of Buffer C to each well with a dipstick.

6. Allow the dipstick to wick up the buffer for 10 minutes.
   Note: Do not allow the dipstick to dry out at any time.

7. Prepare the Activity Buffer by combining Tubes 1, 2, and 3 as per Table 2. Keep at room temperature.

Activity Buffer Volume	Tube 1 (µL)	Tube 2 (µL)	Tube 3 (mL)
5 mL	50	250	4.7
10 mL	100	500	9.4

Table 2. Preparation of Activity Buffer

8. Add 300 µl of Activity Buffer to an empty microplate well for each dipstick used.

9. Remove the wicking pad from the dipstick. Make sure to remove the pad at the junction with the membrane.

10. Place the dipstick in a well with Activity Buffer. The Complex IV (COX) capture mAb is located ~7mm from the bottom of dipstick.

11. Develop for 45 - 60 minutes.
Note: Since this is an end-point reaction, develop all dipsticks for the same time period.

12. Add 300 µl deionized water to an empty well of the microplate.

13. Once the dipstick(s) are developed, add them to the well with deionized water for 10 minutes

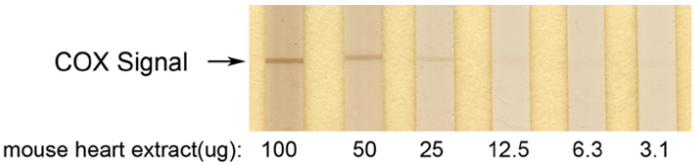
14. Dry the dipstick and measure the signal intensity with a dipstick reader or other imaging system, e.g. flat-bed scanner.
Note: Signal intensity should be measured within 1 hour of drying as extended storage will result in elevated background.

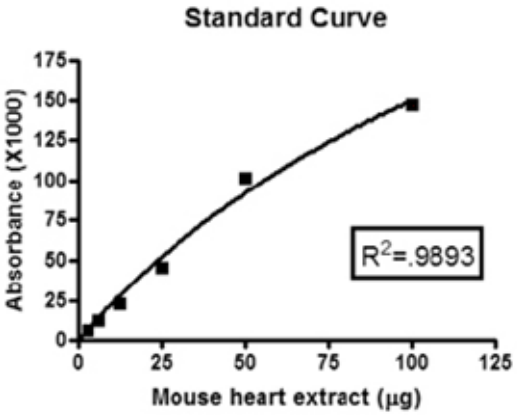
7. Data Analysis

Below is an example using the ab109878 to measure Complex IV activity in mouse protein extracts. Samples were prepared as described in the Sample Preparation section. All data were analyzed using a Dipstick Reader and GraphPad software.

A. Generating a standard curve

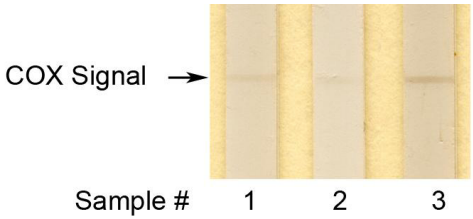
Shown are developed dipsticks from a 1:2 dilution series using a positive control sample and the associated standard curve. Starting material was 100 µg of protein extract.





B. Analysis of samples

Based on the standard curve, 50 µg of protein extract were loaded onto a dipstick for each sample. The figure below shows three developed dipsticks from unknown samples (1-3). The analysis of the signal intensity and interpolation from the standard curve showed that the unknown samples have between 23 - 87% of normal Complex IV activity levels.



Sample #	Abs. (x 1000)	Extrapolated Value (µg)	% control
1	41.3	19.3	38.6
2	25.3	11.3	22.6
3	82.3	43.2	86.4

8. FAQs / Troubleshooting

General troubleshooting points are found at www.abcam.com/assaykitguidelines,

Signal is saturated

It is very important that the amount of sample used is within the working range of the assay (use a best fit line for interpolation). Therefore, it is crucial to determine the working range for your sample type and avoid the region of signal saturation.

Signal is too weak

This occurs when the sample lacks measurable amounts of the protein. Increase the signal by adding more sample protein to another dipstick, or leave the dipstick in the activity solution for longer to maximize the signal

Sample is not wicking up the dipstick

If the dipstick is not gently handled, the nitrocellulose membrane and wicking pad may become separated. Check this junction and simply pinch the dipstick at this point to reconnect the two. Check for proper wicking of the sample.

9. Notes

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