abcam®
discover more

ab109876 – Complex IV Enzyme Activity Dipstick Assay Kit

Instructions for Use

For the quantitative measurement of Complex IV activity in samples from Human and Bovine

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

Version 4 Last Updated: 24 May 2019

Table of Contents

1. Introduction

The Complex IV Human Enzyme Activity Dipstick Assay Kit (ab109876) is used to quantify the activity of the cytochrome *c* oxidase (COX) enzyme complex (EC 1.9.3.1) from human and bovine samples. The isolation of mitochondria is not necessary for the performance of this assay. In this kit the specificity of anti-COX monoclonal antibodies (mAbs) is combined with traditional methods for determining COX enzyme activity by histochemical methods and in-gel activity assays. First, the COX enzyme complex is immnocaptured (i.e immunoprecipated in active form) on the dipstick. Second, the dipstick is immersed in COX activity buffer containing reduced cytochrome *c* and di-amino benzidinetetrachloride (DAB), which serves as the reporter of COX activity (Figure 1A). Immunocaptured COX oxidizes cytochrome *c*, which then oxidizes DAB to form a red-colored precipitate at the COX antibody line on the dipstick. In addition to being quick, the reaction is cyanide-sensitive. The signal intensity of this precipitate corresponds to the level of COX activity in the sample (Figure 1B). The signal intensity is best measured by a dipstick or may be analyzed by another imaging system.

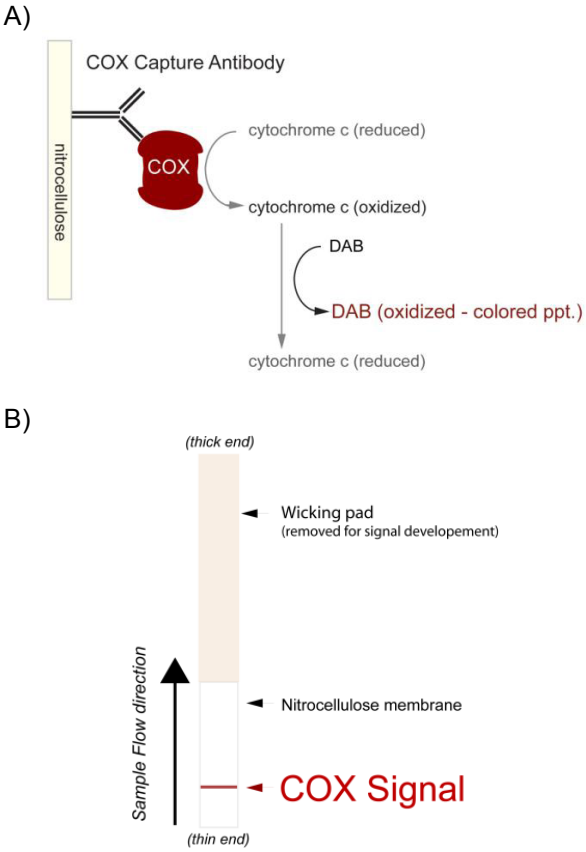


Figure 1. Schematics of the COX Activity reaction and a fully developed COX activity dipstick. ***(A)*** *Mechanism of the COX activity assay; the immunocaptured COX dipstick is immersed in a solution containing reduced cytochrome c and DAB. COX generates oxidized cytochrome c, which in turn oxidizes 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 ~7mm from the bottom of the dipstick.*

2. Assay Summary

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 (~20 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.

Transfer each dipstick to Activity Buffer containing microplate well and incubate for 45 to 60 minutes.

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

Dry dipsticks and measure signal within 1 hour.

3. Kit Contents

Sufficient materials for 90 or 30 measurements.

Item	Quantity	
Dipsticks	90	30
Extraction Buffer (Buffer A)	3 x 15 ml	1 x 15 ml
Blocking Solution (Buffer B)	6 x 1 ml	2 x 1 ml
Wash Buffer (Buffer C)	3 x 1.5 ml	1 x 1.5 ml
Tube 1 (DAB – 100X stock)	3 x 100 µl	1 x 100 µl
Tube 2 (Reduced cytochrome c – 20X stock)	3 x 500 µl	1 x 500 µl
Tube 3 (Buffer for activity assay)	3 x 10 ml	1 x 10 ml
96-well microplate	6	2

4. Storage and Handling

Store dipsticks at room temperature out of direct sunlight in their provided storage containers. High humidity conditions should be avoided. Store Buffers A and C at 4°C or at -20°C for long-term storage. Store Buffer B at -80°C. Store Tube 1 and Tube 2 at -80°C; they can also be aliquoted upon receipt to prevent freeze/thaw cycles. Store Tube 3 at room temperature.

5. Additional Materials Required

- Dipstick reader or other imaging system
- Method for determining protein concentration
- Pipetting devices
- Protease inhibitors

6. Preparation of Samples

The preparation of the sample 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.*

A. Tissue Sample

1. Begin with approximately 25 mg of sample
2. Add 5 volumes/ weight of iced 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 13,000 to 16,000 rpm for 20 minutes at 4°C.

6. Remove the supernatant and determine the protein concentration of the protein extract from the sample. Protein concentration should be approximately 1 mg/ml.

7. Proceed directly to Dipstick Procedure or freeze samples at -80°C.

B. Cell Culture Sample

1. Add 5 volumes/ cell pellet volume of iced 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 microcentrifuge at 13,000 to 16,000 rpm for 20 minutes at 4°C.

4. Save the supernatant and determine the protein concentration of the extracted protein. Protein concentration should be ≥ 1 mg/ml.

5. Proceed directly to Dipstick Procedure or freeze samples at -80°C.

7. Dipstick 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
Fibroblast extract	2 – 100 µg
Bovine heart mitochondria	0.03 – 0.6 µg
Human muscle extract	0.5 – 25 µg

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. Bring sample volume to 25 µl with Buffer A. Add to a well of the microplate.

   Note: if sample concentration is too low, use 100 µl reaction volumes. Make sure to add equal amounts of Buffer B to sample in Buffer A.

3. Follow by adding 25 µl of Buffer B to each well.

4. Gently add a dipstick to the microplate well (place the thin/nitrocellulose end of dipstick down).

5. Allow the sample to wick up into the dipstick. (This step takes 15-25 minutes depending on sample viscosity).

   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.

6. Add 30 µl of Buffer C to each well with a dipstick.

7. Allow the dipstick to wick up the buffer for 10 minutes.

Note: Do not allow the dipstick to dry out at any time.

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

Activity of Activity Buffer Produced	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

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

10. Now, remove the wicking pad from the dipstick. Make sure to remove the pad at the junction with the membrane.

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

12. Develop for 45 - 60 minutes.

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

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

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

15. Dry the dipstick and measure the signal intensity with a dipstick reader or other imaging system, e.g. flat-bed scanner.

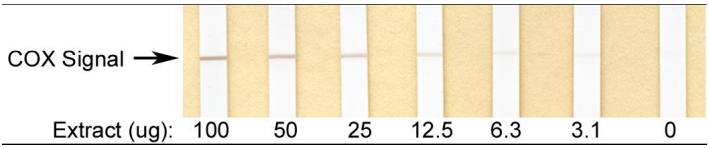
NOTE: Signal intensity should be measured within 1 hour of drying as extended storage will result in elevated background.

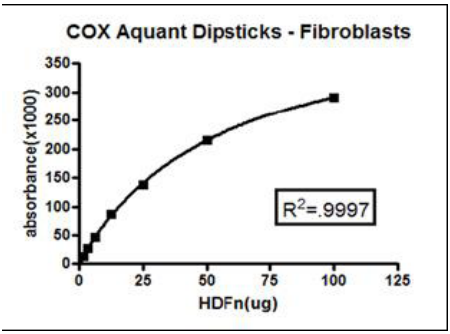
8. Data Analysis

Below is an example using the ab109876 to measure Complex IV activity in fibroblast 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

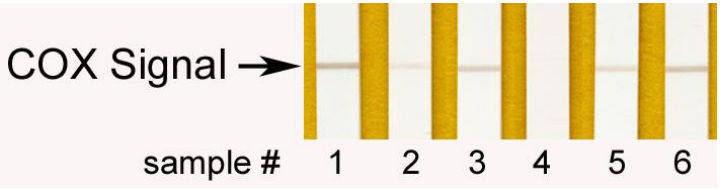
Shown are developed dipsticks from a 1:2 dilution series using a positive control sample and the associated standard curve. Starting material was 100 µg of fibroblast protein extract.





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 five developed dipsticks, a control sample (1) and four unknowns (2-6). The analysis of the signal intensity and interpolation from the standard curve showed that the unknown samples have between 15-61% of normal Complex IV activity levels.



Sample	Signal (Absorbance x1000)	% control
Control (1)	209.8	100
2	52.9	15.2
3	128.2	45.5
4	0	0
5	88.3	27.9
6	156.8	61.1

9. Specificity

Species Reactivity: Human and Bovine

10. Troubleshooting

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 handled gently, 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.

UK, EU and ROW
Email: technical@abcam.com | Tel: +44-(0)1223-696000

Austria
Email: wissenschaftlicherdienst@abcam.com | Tel: 019-288-259

France
Email: supportscientifique@abcam.com | Tel: 01-46-94-62-96

Germany
Email: wissenschaftlicherdienst@abcam.com | Tel: 030-896-779-154

Spain
Email: soportecientifico@abcam.com | Tel: 911-146-554

Switzerland
Email: technical@abcam.com
Tel (Deutsch): 0435-016-424 | Tel (Français): 0615-000-530

US and Latin America
Email: us.technical@abcam.com | Tel: 888-77-ABCAM (22226)

Canada
Email: ca.technical@abcam.com | Tel: 877-749-8807

China and Asia Pacific
Email: hk.technical@abcam.com | Tel: 400 921 0189 / +86 21 2070 0500

Japan
Email: technical@abcam.co.jp | Tel: +81-(0)3-6231-0940

www.abcam.com | www.abcam.cn | www.abcam.co.jp