

ab109720 – Complex I Enzyme Activity Dipstick Assay Kit

Instructions for Use

For the accurate quantitative measurement of Complex I activity in human, bovine, mouse and rat samples.

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

Note: The reconstitution instructions for the NADH component was updated on 24 February 2017. Please read the Reagent Preparation instructions carefully before proceeding.

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1. BACKGROUND

Abcam's Complex I Enzyme Activity Dipstick Assay kit is designed for the accurate quantitative measurement of Complex I activity in human, bovine, mouse and rat samples.

In this assay the specificity of anti-Complex I monoclonal antibodies (mAbs) is combined with the well-characterized Complex I in-gel activity assay that is not rotenone sensitive. First, Complex I is immunocaptured (i.e. immuno-precipitated in active form) on the Dipstick. Second, the Dipstick is immersed in Complex I activity buffer solution containing NADH as a substrate and nitrotetrazolium blue (NBT) as the electron acceptor (Figure 1A). Immunocaptured Complex I oxidizes NADH and the resulting H⁺ reduces NBT to form a blue-purple precipitate at the Complex I antibody line on the Dipstick. The signal intensity of this precipitate corresponds to the level of Complex I enzyme activity in the sample. Combined with Dipstick assay kit for measuring Complex I quantity (ab109722 for Human samples; ab109875 for rodent samples), it is possible to determine the relative specific activity of immunocaptured Complex I. The signal intensity is best measured by a Dipstick reader or may be analyzed by a standard imaging system. The isolation of mitochondria is not necessary for the performance of this assay.

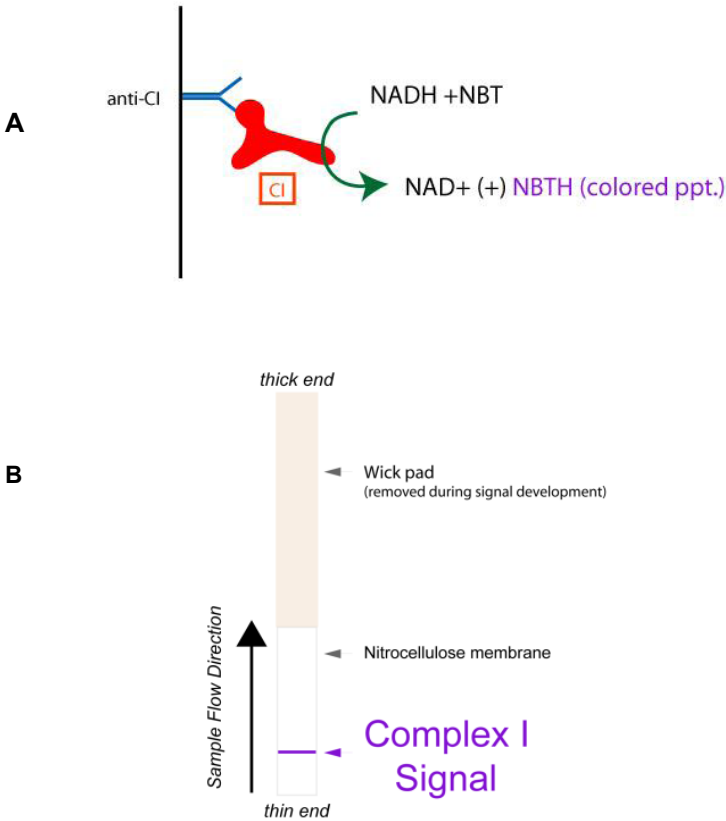
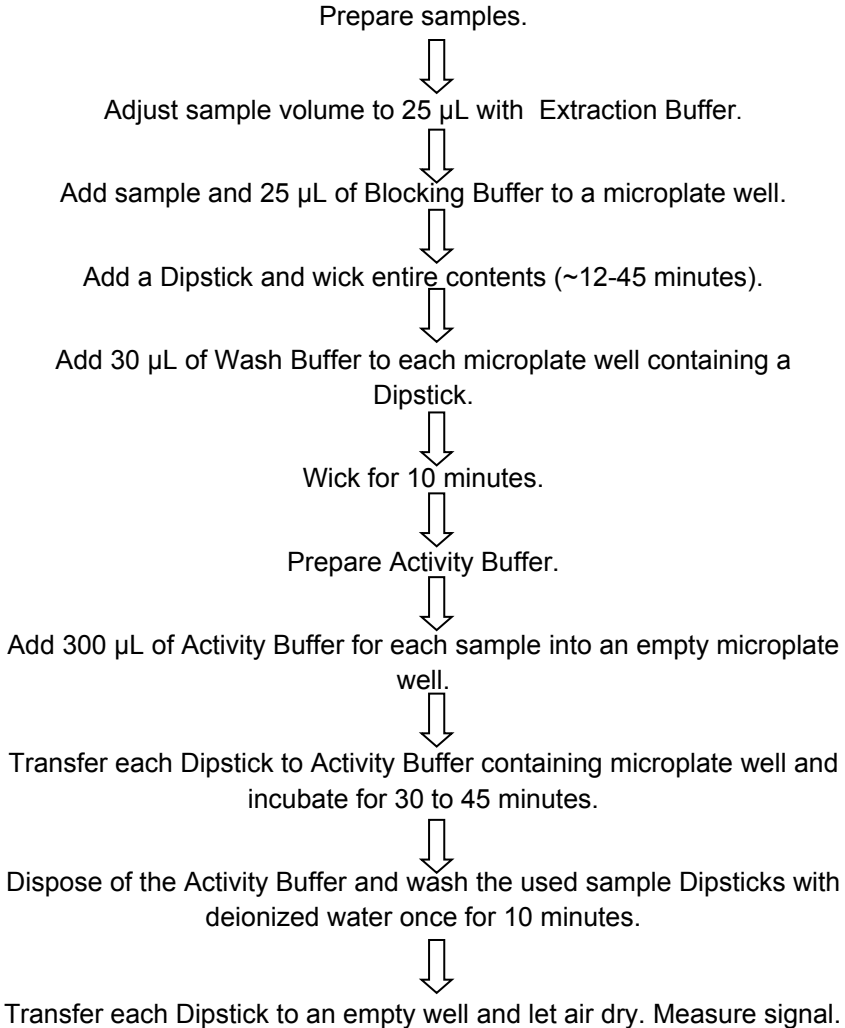


Figure 1. Schematics of the Complex I activity Dipstick reaction and a fully developed Dipstick. (A) Mechanism of the assay reaction; the Dipstick with the immunocaptured Complex I is immersed in a solution containing NADH and NBT. Complex I oxidizes NADH which in turn reduces NBT to form a bluish-purple precipitate at the antibody line. **(B)** A developed Complex I Activity Dipstick with the wicking pad removed. Note: The anti-Complex I mAb is located ~7 mm from bottom of Dipstick. This reaction is not rotenone sensitive.

2. ASSAY SUMMARY



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. Modifications to the kit components or procedures may result in loss of performance.

4. STORAGE AND STABILITY

Store kit at either 4°C or room temperature (RT) immediately upon receipt. See table below for specific instructions.

Refer to list of materials supplied for storage conditions of individual components. Observe the storage conditions for individual prepared components in section 9.

5. MATERIALS SUPPLIED

Item	Amount	Storage Condition (Before Preparation)
Complex I Enzyme Activity Dipsticks	48	4°C or RT
Extraction Buffer	15 mL	4°C
10X Blocking Buffer	400 µL	4°C
Wash Buffer	2 mL	4°C
NBT (lyophilized)	10 mg	4°C
NADH (lyophilized)	2 mg	4°C
Reaction Buffer	15 mL	4°C or RT
96-Well Microplate	2 x 96 Wells	4°C or RT

6. MATERIALS REQUIRED, NOT SUPPLIED

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

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

- Dipstick reader or other imaging system.
- Method for determining protein concentration (BCA assay recommended).
- Deionized water.
- Multi and single channel pipettes.
- Tubes for standard dilution.
- Optional: Phenylmethylsulfonyl Fluoride (PMSF) (or other protease inhibitors).

7. 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.

8. TECHNICAL HINTS

- 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.
- As a guide, typical ranges of sample concentration for commonly used sample types are shown below in Sample Preparation (section 10).
- All samples should be mixed thoroughly and gently.
- Avoid multiply freeze/thaw of samples.

9. REAGENT PREPARATION

Equilibrate all reagents and samples to room temperature (18-25°C) prior to use.

9.1 **2X Blocking Buffer**

Prepare 2X Blocking Buffer by adding 1.6 mL distilled water to 400 μ L 10X Blocking Buffer.

9.2 **100X NADH**

Immediately prior to use, prepare 100X NADH by adding 0.2 mL of distilled water to NADH vial. Vortex thoroughly until dissolved.

9.3 **20X NBT**

Prepare 20X NBT by adding 1 mL distilled water to NBT vial. Vortex thoroughly until dissolved (this may take several minutes). 20X NBT should be prepared immediately prior to use.

9.4 **1X Activity Buffer**

Prepare the 15 mL 1X Activity Buffer by combining 750 μ L 20X NBT, 150 μ L 100X NADH and 14.1 mL Reaction Buffer.

- Unused Reaction Buffer should be stored at 4°C, while any unused 100X NADH and 20X NBT should be aliquoted and stored at -80°C.

10. SAMPLE PREPARATION

General Sample information:

- This kit can be used with previously stored material. However, note that storage conditions and duration could affect results.
- The Extraction Buffer provided with this kit, should only be used with mammalian expression systems. This buffer can be supplemented with phosphatase inhibitors, PMSF and protease inhibitor cocktail prior to use. Supplements should be used according to manufacturer's instructions.
- Samples should be assayed immediately after collection. Samples that cannot be assayed immediately should be stored at -80°C

TYPICAL SAMPLE DYNAMIC RANGE -

Typical working ranges	
Sample Type	Range (µg)
Fibroblast Extract	1 – 30
Skeletal Muscle Extract	0.5 – 15
Bovine Heart Mitochondria	0.01 – 5
Mouse Tissue Extract	0.05 – 5

10.1. Preparation of extracts from cell pellets

- 10.1.1. Collect non adherent cells by centrifugation or scrape to collect adherent cells from the culture flask. Typical centrifugation conditions for cells are 500 x g for 5 minutes at 4°C.
- 10.1.2. Add 10 volumes of Extraction Buffer to the cell pellet and mix e.g. if the size of the cell pellet displaces a volume of 50 µL, then add 500 µL of Extraction Buffer.
- 10.1.3. Incubate on ice for 20 minutes, mixing intermittently. Centrifuge at 18,000 x g for 20 minutes at 4°C.

10.1.4. Transfer the supernatants into clean tubes and discard the pellets. Assay samples immediately or aliquot and store at -80°C . The sample protein concentration in the extract should be quantified using a protein assay.

10.2. Preparation of extracts from tissue homogenates

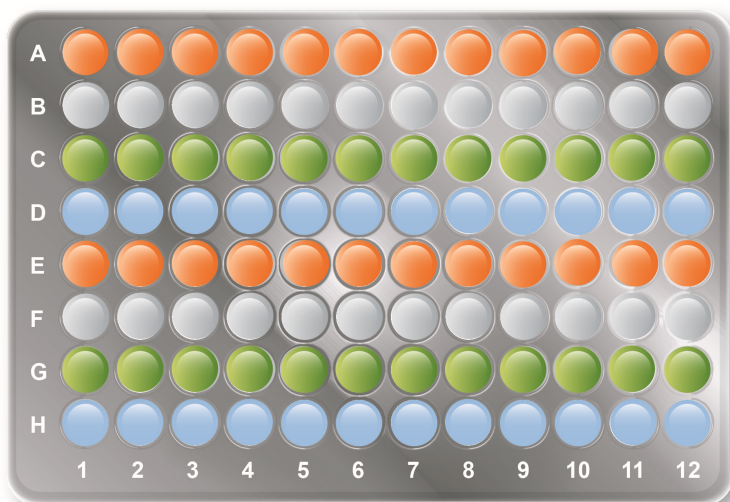
10.2.1. Tissue lysates are typically prepared by homogenization of tissue that is first minced and thoroughly rinsed in PBS to remove blood (dounce homogenizer recommended).

10.2.2. Homogenize 100 to 200 mg of wet tissue in 500 μL of Extraction Buffer. For lower amounts of tissue adjust volumes accordingly.

10.2.3. Incubate on ice for 20 minutes. Centrifuge at $18,000 \times g$ for 20 minutes at 4°C . Transfer the supernatants into clean tubes and discard the pellets. Assay samples immediately or aliquot and store at -80°C . The sample protein concentration in the extract may be quantified using a protein assay.

11. PLATE PREPARATION

- The plate layout below illustrates the wells to which the user should add the samples, Wash Buffer, Activity Buffer and water. The direction of the movement of the Dipsticks throughout the assay is also indicated. Two blank plates are provided for the user to accommodate use for 48 Dipsticks.
- Two blank plates are provided for the user to accommodate use for 48 Dipsticks



12. ASSAY PROCEDURE

- **Equilibrate all materials and prepared reagents to room temperature prior to use.**
- **It is recommended to assay all standards, controls and samples in duplicate.**
- **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 typical sample dynamic range table in section 10, generate a standard curve using a positive control sample.**

12.1. Prepare all reagents, working standards, and samples as directed in the previous sections.

12.2. Dilute samples to a total volume of 25 μL in Extraction Buffer. The amount of sample used should correspond toward the high end of the user generated standard curve (~3/4 of the high end).

12.3. Add 25 μL of sample in Extraction Buffer and 25 μL of 2X Blocking Buffer to a microplate well and mix (see plate layout, section 11).

Note: If the protein concentration is too low, 100 μL reaction volumes are possible. However, be sure to keep the ratio of sample in Extraction Buffer to Blocking Buffer constant.

12.4. Gently add a Dipstick to the microplate well (place the thin/nitrocellulose end of Dipstick into the well). The Dipstick must reach the bottom of the well.

12.5. Allow the sample to wick up into the Dipstick. This step takes 15 - 45 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.

12.6. Add 30 μL of Wash Buffer to appropriate wells (see plate layout, section 11).

- 12.7. Transfer Dipstick to well containing Wash Buffer.
- 12.8. Allow the Dipstick to wick up the Wash Buffer (~10 minutes). During this step, proceed with step 12.9.
Note: Do not allow the Dipstick to dry out at any time.
- 12.9. Add 300 μ L of 1X Activity Buffer to an empty microplate well for each Dipstick used (see plate layout, section 11).
- 12.10. Remove the wicking pad from the Dipstick. Make sure to remove the pad at the junction with the membrane.
- 12.11. Place the Dipstick into the microplate well containing 1X Activity Buffer. The Complex I capture mAb is located ~7mm from the bottom of Dipstick.
- 12.12. Allow the Dipstick to develop for 30 - 45 minutes.
Note: Since this is an end-point reaction, expose all samples to 1X Activity Buffer for the same period of time
- 12.13. Add 300 μ L deionized water to an empty microplate well for each Dipstick used (see plate layout, section 11).
- 12.14. Once the Dipsticks are developed add them to the well with deionized water for 10 minutes
- 12.15. Remove the Dipstick from the well and allow to dry.
- 12.16. Measure the signal intensity with a Dipstick reader or other imaging system, e.g. flat-bed scanner.

13. TYPICAL DATA

Below is an example using the ab109720 to measure Complex I activity in Human fibroblast samples. Samples were prepared as described in the Sample Preparation section. All data were analyzed using a Dipstick Reader and GraphPad software.

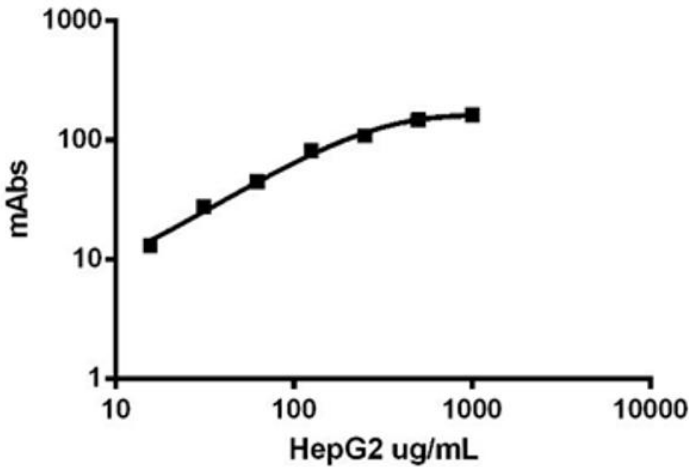
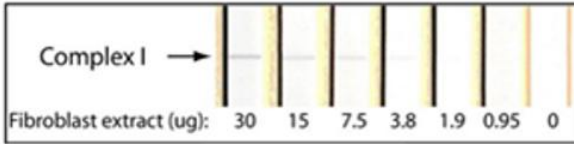
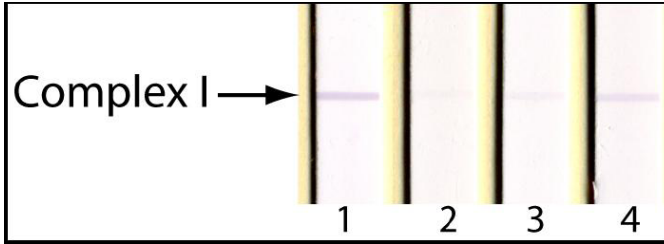


Figure 2. Shown are developed Dipsticks from a 1:2 dilution series using a positive control sample and the associated standard curve. Starting material was 30 µg of fibroblast protein extract.

Analysis of samples



Control	Abs (x1,000) average	% Normal
1	80.2	100
2	13.8	14.02
3	29.3	31.13
4	53.3	60.86

Figure 3. Based on the standard curve, 15 µg of protein extract were loaded onto a Dipstick for each sample. The figure below shows four developed Dipsticks, a control sample (1) and three unknowns (2-4). The analysis of the signal intensity and interpolation from the standard curve showed that the unknown samples have between 14 - 61% of normal Complex I activity levels.

14. ASSAY SPECIFICITY

This kit detects Complex I in **Human, bovine, mouse and rat samples only**.

15. TROUBLESHOOTING

Problem	Solution
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.

16. NOTES

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