



ab109722 –

**Complex I Human
Protein Quantity
Dipstick Assay Kit**

Instructions for Use

For the quantitative measurement of Complex I Protein Quantity in samples from Human and Bovine

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

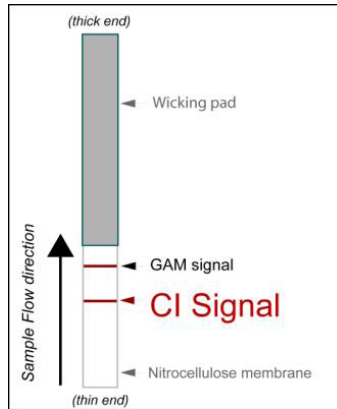
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1. Introduction

Abcam's ab109722 Complex I Human Protein Quantity Dipstick Assay Kit is designed for the accurate quantitative measurement of the Complex I enzyme complex in Human and bovine. Based on the immunologic sandwich assay, the kit utilizes two monoclonal antibodies (mAbs) specific to different antigens present on the Complex I enzyme complex. One antibody is immobilized on the nitrocellulose membrane in a thin line perpendicular to the length of the dipstick, while the other is gold-conjugated and combined with the sample mix. The sample contents containing the gold-conjugated mAb wick past the mAb immobilized on the dipstick. When assembled Complex I is present in the sample, a red line appears at the site of the anti-Complex I antibody line (Figure 1). The signal intensity is directly related to the amount of Complex I in the sample. The signal intensity is best measured by a dipstick reader or may be analyzed by another imaging system. To identify defects in Complex I that do not affect enzyme assembly combine this assay with ab109720 to determine the relative specific activity of Complex I.

A)



B)

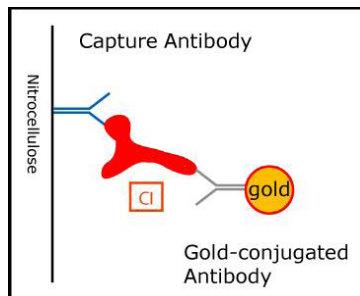


Figure 1. Schematics of a developed Complex I quantity dipstick and the interaction between the mAbs and Complex I. (A) A finished dipstick shows a strong signal for Complex I (bottom band) and the positive control (goat anti-mouse antibody (GAM)) which is used to verify completion of the reaction (i.e. flow of the gold conjugated antibody and sample contents past the capture antibody line). **(B)** The antibody sandwich diagram showing the interaction between Complex I and the two monoclonal antibodies in this assay.

2. Assay Summary

Prepare samples.



Prepare Standard Curve.



Dilute samples to a volume of 25 μl in Extraction Buffer.



Add 25 μl of 2X Blocking Buffer to sample and transfer all 50 μl to dried gold-conjugated antibody microplate well.



Mix contents to re-suspend antibody.



Add dipstick to microplate well and let sample wick up onto dipstick.



Add 30 μl of Wash Buffer to sample and let buffer wick up onto dipstick.



Let dipstick air dry.



Measure signal.

3. Kit Contents

Sufficient materials for 48 measurements.

Item	Quantity
Dipsticks	48
Gold-conjugated antibody (dried in microplate wells)	48 wells
Extraction buffer	15 ml
10X Blocking buffer	400 μ L
Wash buffer	2 ml

4. Storage and Handling

All components are stable in their provided containers at room temperature out of direct sunlight. After diluting the 10X Blocking Buffer to 2X, store at 4°C. For long-term storage, all buffers can be stored at 4°C.

5. Additional Materials Required

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

6. Preparation of Samples

Note: *Samples must be kept on ice.*

A. Tissue Sample

1. Start with approximately 25 mg of tissue sample. Add 10 volumes of Extraction Buffer per microgram of sample (e.g. if the sample weighs 50 mg, add 500 μ l of Buffer).
2. Homogenize the sample.
3. Keep on ice for 20 minutes, mixing intermittently.
4. Spin the cell extract in a microcentrifuge at 13,000-16,000 rpm at 4°C for 20 minutes to pellet cellular debris.
5. Transfer supernatant to a new tube and determine protein concentration (e.g. BCA assays are

recommended as the detergent in Extraction Buffer does not affect the reading in the assay).

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

B. Cell Culture Sample Preparation

1. Start with a cell pellet. Add 10 volumes of Extraction Buffer to the cell pellet and mix (e.g. if the total sample volume displaces 50 μl of volume, then add 500 μl of Buffer).
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. Transfer supernatant to a new tube and determine protein concentration (e.g. BCA assays are recommended as the detergent in Extraction Buffer does not affect the reading in the assay).
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 (μg)
Human Fibroblasts	0.5 – 20
PBMC	0.5 – 20
Lymphoblastoid	0.25 – 10
Fat	2 – 40
Bovine heart mitochondria	5 – 300

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. Add Extraction Buffer to the sample to bring volume up to 25 μl .

3. Make 2X Blocking Buffer by adding 1.6 mL H₂O to 0.4 mL 10X Blocking Buffer.
4. Add the sample and 25 µL of 2X Blocking Buffer to a microplate well of dried gold-conjugated antibody, for a total sample volume of 50 µL. Incubate the sample for 5 minutes, allowing the gold-conjugate to hydrate.
5. Re-suspend the dried gold-conjugated secondary antibody using a pipette.
6. Gently add a dipstick to the microplate well (place the thin/nitrocellulose end of dipstick down). The dipstick must reach the bottom of the well.
7. Allow the sample wick up onto the dipstick until the entire sample volume has been absorbed (the positive control band typically appears within 2 minutes).

A 50 µl sample should be wicked up completely in 20 minutes; more viscous extracts may take longer. **The dipstick must absorb the entire sample volume before Wash Buffer is added.**

8. Add 30 µL of Wash Buffer and let the entire buffer volume wick up onto the dipstick.

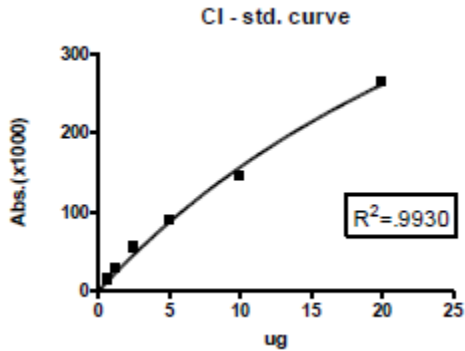
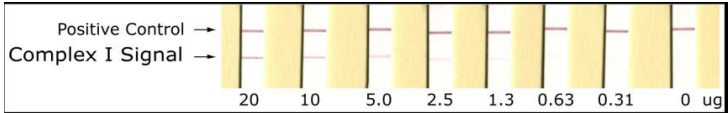
9. Leave dipstick to air-dry (requires approximately 20 minutes to air dry, or 10 minutes in an incubator at 37°C).
10. Measure the signal intensity with a dipstick reader or other imaging system, e.g. flat-bed scanner.

8. Data Analysis

Illustrated is an experiment using the ab109722 assay kit to quantify Complex I levels using various concentration of human fibroblast extract. Samples were prepared as described in this protocol. All data were analyzed using a Dipstick Reader and GraphPad software.

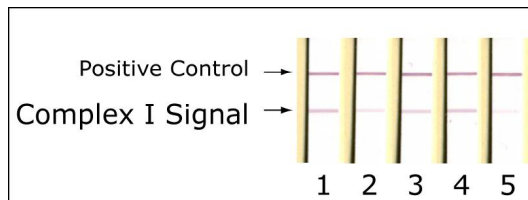
A. Generating a standard curve

Shown is a 1:2 dilution series using a positive control sample (see Table 1 for recommended protein ranges). Approximately 7 to 8 dipsticks are suitable for covering the entire working range and the blank for background levels. In this example the dilution series starts with 20 µg of fibroblast extract. A one-site hyperbola line was generated for best-fit analysis using GraphPad.



B. Analysis of samples

Based on the above standard curve values, 13 μg of protein extract were loaded to each dipstick for the control sample (1) and the four unknown samples (2-5).



Control	Abs. (x1000) average	% error	% normal*
1	194.4	6.18355	100
2	78.1	2.40067	33.42372
3	128.05	1.20034	59.06275
4	171.7	3.20089	84.97313
5	41.35	1.05484	16.80446

Using GraphPad software, the signal intensity from the standard curve was interpolated and the quantity of Complex I in samples 1-5 was determined. Based on the above analysis, the unknown samples had 16% and 85% of Complex I levels compared to the control.

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 (a best fit line for interpolation as generated with the GraphPad program). Therefore, it is crucial to know the working range for the 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.

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