



ab109880

**MetaPath™ Fatty Acid
Oxidation 4-Plex Dipstick
Array**

Instructions for Use

For the measurement of 4 mitochondrial protein parameters in human samples.

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

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1. Introduction

ab109880 is a rapid and simple test for determining four mitochondrial protein parameters: Two key fatty acid oxidation acyl dehydrogenases SCHAD (short chain hydroxyacyl CoA dehydrogenase), and MCAD (medium chain acyl CoA dehydrogenase), and the TFP (trifunctional protein). The kit determines whether these enzymes have been up-regulated or down-regulated in human samples as a result of drug interactions or conditions such as oxidative stress and genetic disease. The kit also measures the levels of a key mitochondrial iron regulatory protein frataxin which is also the cause of Friedreich's ataxia (FA) the most prevalent inherited ataxia.

The method is based on an immunological sandwich assay (see Figure 1). Each enzyme is bound by two unique enzyme specific monoclonal antibodies. To accomplish this, one antibody is gold labeled and mixed with the sample. The other is embedded in the strip in one of four vertically separated zones. As the sample wicks past each zone the corresponding enzyme (now gold labeled) is trapped, this results a red colored band which is directly proportional in intensity to the amount of each enzyme captured.

The assay works on very small amounts of sample material, including cheek swabs or single drops of blood, and results can be

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generated in under 30 minutes. The assay also works on cultured cells and is suitable for any situation in which speed and simplicity are required.

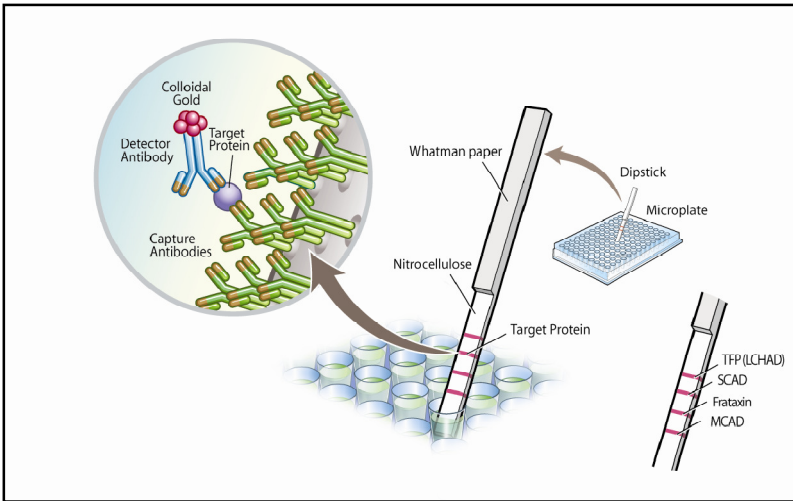


Figure 1. ab109880 is a lateral flow/dipstick device measuring the iron regulatory protein frataxin and the mitochondrial fatty acid oxidation enzymes SCHAD, MCAD and trifunctional protein TFP (including LCHAD).

Standard curves of TFP, SCHAD, MCAD and frataxin levels are generated by the user with a control dilution series of normal sample material. The signal intensities of the four bands on the dipsticks are measured by a dipstick reader or may be analyzed by another imaging system. The levels of all 4 enzymes in an experimental sample are then measured by interpolating their signal intensities into the standard curves. The levels and ratios of the enzymes in experimental samples are then compared with the control dilution series.

2. Assay Summary

Prepare samples.



Prepare Standard Curve.



Add 25 μ l of 2X Buffer B to a well with dried, gold-conjugated mAb.



After 5 minutes, add 25 μ l of sample in Buffer A to the above well.



Mix contents to re-suspend gold conjugated mAb.



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



Add 50 μ l of Buffer C to well and wick completely.



Dry dipstick.



Measure signal.

3. Kit Contents

Sufficient materials for 90 or 30 measurements.

Item	Quantity	
Dipsticks	90	30
Gold-conjugated antibody (dried in microplate wells)	90 wells	30 wells
Buffer A (Extraction buffer)	45 ml	15 ml
Buffer B (10X Blocking buffer)	1.2 ml	0.4 ml
Buffer C (Wash buffer)	6 ml	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. Proteases inhibitors are not provided. Enough Buffer A is provided (Extraction Buffer) for preparation of 20 samples using 500 µl /sample.*

A. Tissue Sample Preparation

1. Start with approximately 25 mg of tissue sample. Add 10 volumes of Buffer A per microgram of sample (e.g. if the sample weighs 50 mg, add 500 µl of Buffer A).
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 Buffer A 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 5-10 volumes of Buffer A to the cell pellet and mix (e.g. if the total sample volume displaces 50 µl of volume, then add 250-500 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 to pellet cellular debris.
4. Transfer supernatant to a new tube and determine protein concentration (e.g. BCA assays are recommended as the detergent in Buffer A 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. We recommend performing a 1:2 serial dilution with 1 part Buffer A to 1 part Buffer B in a total volume of 50 µl.

Sample Type	Suggested Working Range (µg)
Fibroblasts	0.1 – 10 µg
HeLa cells	0.1 – 10 µg
HepG2 cells	0.05 – 5 µg
Muscle extract (Human)	0.1 – 1.5 µg

Table 1. *Suggested working range for different sample types*

For Individual Dipstick Reactions:

1. Make 2X Buffer B by adding 1.6 mL H₂O to 0.4 mL 10X Buffer B.
2. Add 25 µL of 2X Buffer B to each well of gold conjugated mAb as necessary.
3. Allow gold conjugated mAb to re-hydrate for 5-10 minutes.
4. Now, add 25 µL of sample in Buffer A for at total volume of 50 µL and mix to homogeneity.

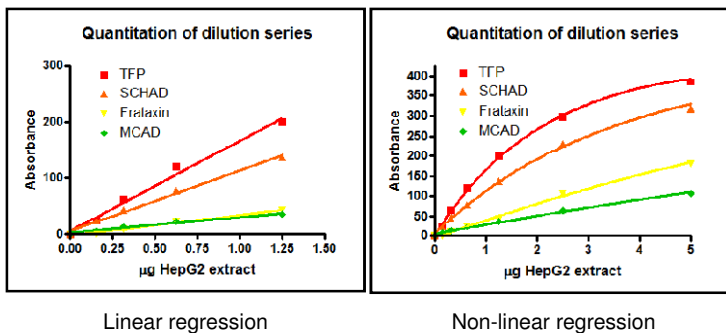
5. Gently add a dipstick to the well with the thin end down (see Figure 1). The contents will immediately begin wicking up the dipstick.
6. Wick the entire contents before proceeding to the wash step: the positive control band typically appears within 1-2 minutes. A 50 μ l sample should wick completely in 12-20 minutes. More viscous extracts may take longer.
7. Add 50 μ l of Buffer C and wick completely.
8. Dry the dipstick(s) before analysis (~20 minutes to air dry, or ~10 minutes at 37°C).
9. Measure the signal intensity with a dipstick reader or other imaging system, e.g. flat-bed scanner.

8. Data Analysis

The experiment shown below shows a standard curve for each analyte generated from a dilution series of normal HepG2 cells. HepG2 whole cell extracts were prepared as described in this protocol. All data were analyzed using and GraphPad software.

A. Generating a standard curve

Below we show a typical standard curve for the ab109880 Dipstick using HepG2 cell extract (see Table 1). We suggest using 7 to 8 dipsticks for covering the working range. In this example, the maximum sample load should be 1.25 µg linear regression above which non-linear regression should be used.



Intra-assay and inter-assay CVs are typically ≤10%.

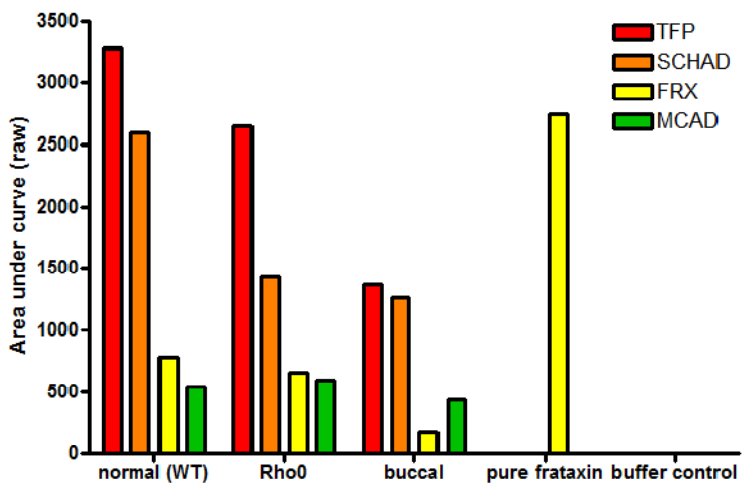
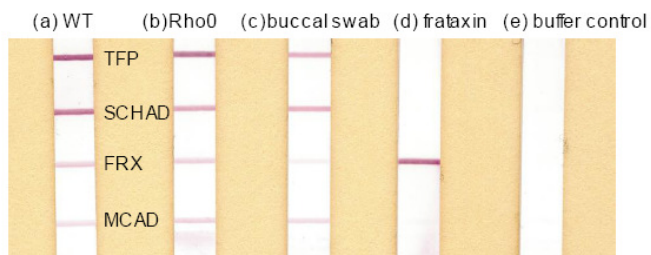
Example: A 2.5 µg HepG2 sample was analyzed using ab109880 and bands quantified in absorbance units by a dipstick reader.

	Average	n	c.v. (%)
TFP	308	4	2.6
SCHAD	228	4	2.6
Frataxin	98	4	7.4
MCAD	58	4	6.5

B. Measurement using desktop scanner: Analysis of samples with mitochondrial deficiencies show signal specificity.

Shown below are examples of dipsticks from (a) normal cells (b) rho0 cells which lack mtDNA and hence the respiratory chain but are otherwise well represented in these FAO enzymes (c) a sample collected by a buccal swab collection (d) pure frataxin and (e) buffer control with containing no sample. Data from these sticks was collected using a simple flatbed scanner and ImageJ software.

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9. Specificity

Species Reactivity: Human

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 (see Table 1). Determination of the working range is recommended for the sample in case of signal saturation.

Signal is too weak

This occurs when the sample lacks measurable amounts of the protein. To increase signal add more sample to another dipstick.

Abcam in the USA

Abcam Inc
1 Kendall Square, Ste B2304
Cambridge,
MA 02139-1517
USA

Toll free: 888-77-ABCAM (22226)

Fax: 866-739-9884

Abcam in Europe

Abcam plc
330 Cambridge Science Park
Cambridge
CB4 0FL
UK

Tel: +44 (0)1223 696000

Fax: +44 (0)1223 771600

Abcam in Japan

Abcam KK
1-16-8 Nihonbashi
Kakigaracho,
Chuo-ku, Tokyo
103-0014
Japan

Tel: +81-(0)3-6231-094

Fax: +81-(0)3-6231-0941

Abcam in Hong Kong

Abcam (Hong Kong) Ltd
Unit 225A & 225B, 2/F
Core Building 2
1 Science Park West Avenue
Hong Kong Science Park
Hong Kong

Tel: (852) 2603-682

Fax: (852) 3016-1888