PROTOCOL

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Frataxin Protein Quantity Dipstick Assay Kit

MSF31

Rev.2

DESCRIPTION

Frataxin Protein Quantity Dipstick Assay Kit

Sufficient materials for 30 (MSF31-30) or 90 (MSF31-90) measurements.

Kit Contents:

Item	MSF31-30	MSF31-90
Dipsticks	30	90
Gold-conjugated antibody (dried in microplate wells)	30 wells	90 wells
Buffer A* (Extraction buffer)	15 mL	45 mL
Buffer B* (Blocking buffer)	1 mL	3 mL
Buffer C* (Wash buffer)	1 mL	3 mL

Storage:

Store dipsticks and microplate with gold-conjugated antibody at room temperature out of direct sunlight in their provided containers. High humidity conditions should be avoided. Store Buffers A, B, and C at 4°C, or at -20°C for long-term storage. **Buffers A, B, and C are interchangeable with Buffers A, B, and C from other Quantity Dipstick Assay Kits (MS131, MS133, MS431, MSP31).*

INTRODUCTION

The Frataxin Protein Quantity Dipstick Assay Kit (MSF31) is used to rapidly quantify frataxin protein levels from human sample materials. Purification of mitochondria is not necessary for the performance of this assay. Based on the immunologic sandwich assay, the kit utilizes two monoclonal antibodies (mAbs) specific to different antigens present on the mature form of frataxin. One antibody is immobilized on the nitrocellulose membrane of the dipstick in a thin line perpendicular to the length of the dipstick while the other is gold-conjugated which gives a visual signal (Figure 1A). When frataxin is present in the sample, a red-colored line appears on the dipstick at the site of the anti-frataxin mAb immobilized on the membrane. The signal intensity is directly related to the level of frataxin in the sample. The signal intensity is best measured by a dipstick reader (MitoSciences' MS1000) or may be analyzed by another imaging system.

A) B)

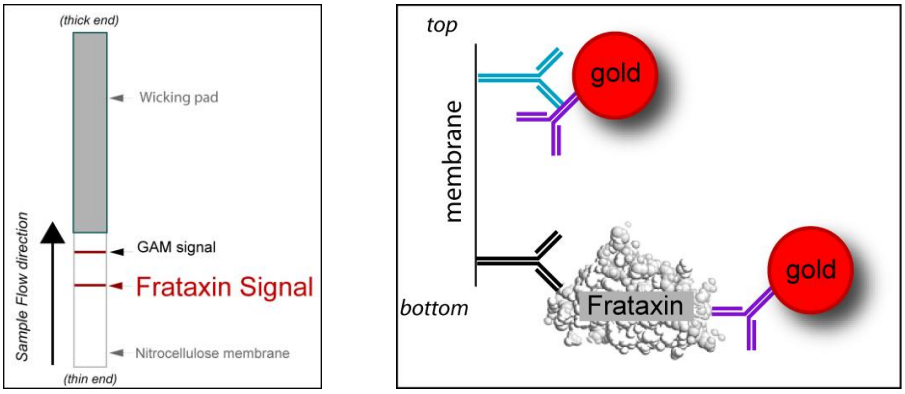


Figure 1: Schematics of a developed frataxin dipstick and the immunologic binding.
(A) *A finished dipstick showing a strong signal for frataxin (bottom band) and the positive control (goat anti-mouse antibody (GAM)), which is used to verify completion of the reaction (i.e. flow of the gold conjugated antibody and sample contents past the anti-frataxin antibody line).* ***(B)*** *The antibody sandwich diagram showing the interaction between frataxin and the immobilized mAb on the membrane (black mAb) and the gold-conjugated mAb (purple mAb) in solution. This also demonstrates the interaction between the GAM antibody (blue) and the gold-conjugated anti-frataxin mAb (purple).*

ADDITIONAL MATERIALS REQUIRED

- Dipstick reader (MitoSciences' MS1000) or other imaging system
- Method for determining protein concentration
- Pipetting devices
- Protease inhibitors

DIPSTICK ASSAY PROTOCOL

A. Sample Preparation

Note: *Samples must be kept on ice. Protease inhibitors are not provided.*

1. Tissue Sample Preparation

    a. 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).

    b. Homogenize the sample.

    c. Keep on ice for 20 minutes, mixing intermittently.

    d. Spin the cell extract in a microcentrifuge at 13,000-16,000 rpm at 4°C for 20 minutes to pellet cellular debris.

    e. 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).

    f. Proceed directly to Part B of the Protocol or freeze samples at -80°C.

2. Cell Culture Sample Preparation

    a. Start with a cell pellet. Add 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 500 µL of Buffer A).

    b. Keep on ice for 20 minutes, mixing intermittently.

    c. Spin the cell extract in a microcentrifuge at 13,000-16,000 rpm at 4°C for 20 minutes to pellet cellular debris.

    d. 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).

    e. Proceed directly to Part B of the protocol or freeze samples at -80°C.

3. Whole Blood Preparation

    a. Beginning with fresh or frozen whole blood, add 1 part whole blood with 3 parts Buffer A.

    b. Keep the sample on ice for 15 minutes.

    c. Spin the cell extract in a microcentrifuge at 13,000-16,000 rpm at 4°C for 20 minutes to pellet cellular debris.

    d. 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).

    e. Proceed directly to Part B of the protocol or freeze samples at -80°C.

B. 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
Human fibroblasts	0.5 - 10 µg
PBMC	0.5 - 10 µg
Lymphoblastoid cells	0.15 - 4 µg
Muscle tissue	0.3 - 5 µg
Whole blood	0.3 - 9 µL

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 Buffer A to the sample to bring volume up to 25 µL.

3. Add the sample and 25 µL of Buffer B 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.

4. Re-suspend the dried gold-conjugated secondary antibody using a pipette or gently vortex.

5. 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.

6. 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 15-20 minutes; more viscous extracts may take longer. **The dipstick must absorb the entire sample volume before Buffer C is added**.

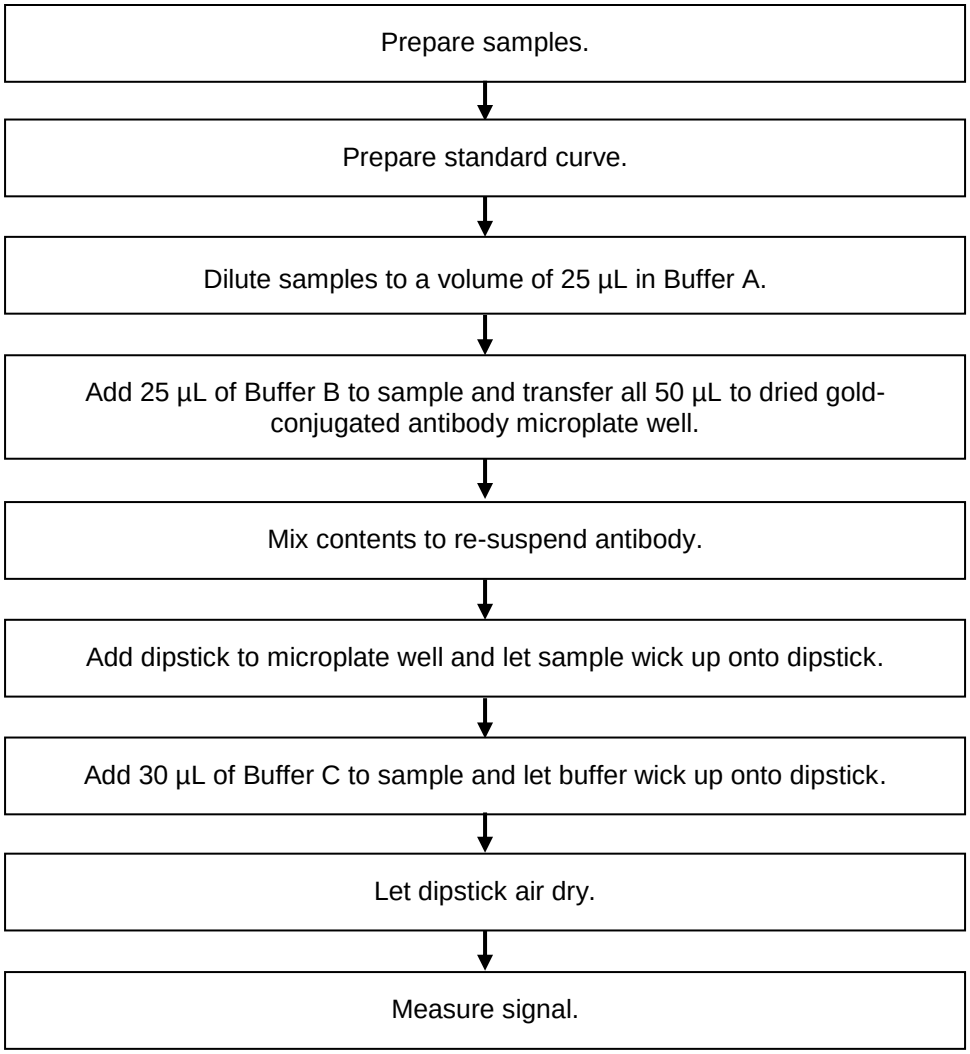
7. Add 30 µL of Buffer C and let the entire buffer volume wick up onto the dipstick.

8. Leave dipstick to air-dry (requires approximately 20 minutes to air dry, or 10 minutes in an incubator at 37°C).

9. Measure the signal intensity with a dipstick reader (MitoSciences' MS1000 Dipstick Reader) or other imaging system, e.g. flat-bed scanner.

FLOW CHART

For quick reference only. Be completely familiar with the previous details of this protocol before performing the assay.

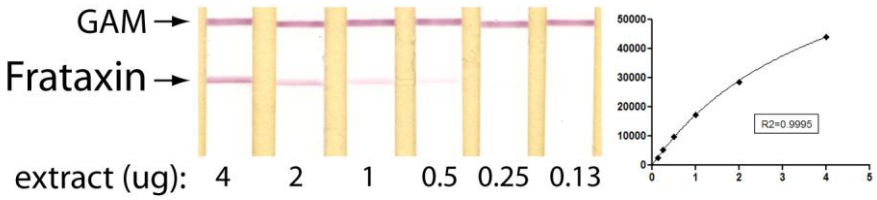


EXAMPLE EXPERIMENT

Illustrated is an experiment using the MSF31 assay kit to quantify frataxin levels from β -lymphocyte cell culture samples derived from control individuals and Friedreich's Ataxia (FA) patients*. Samples were prepared as described in this protocol. All data were analyzed using MitoSciences' Dipstick Reader (MS1000) and GraphPad software.

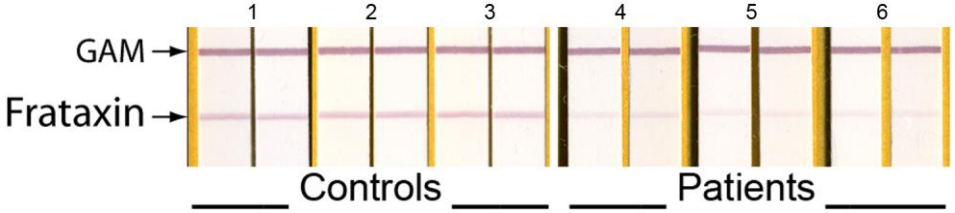
Step 1. Generating a standard curve

Shown is a 1:2 dilution series using a positive control sample (see Table 1 for recommended protein ranges). Approximately 6 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 4 µg of control sample. A one-site hyperbola line was generated for best-fit analysis using GraphPad.



Step 2. Analysis of samples

Based on the above standard curve values, of protein extract for the controls and FA patient samples (note: for a statistical analysis it is preferred to use two dipsticks for each sample; intra-assay CV's are typically <=10%). For this analysis, the FA patients had between 550 - 925 GAA repeats on the smaller allele.



Using GraphPad software, the signal intensity from the standard curve was interpolated and the relative amount of frataxin in the patients, as compared to the controls, was determined. Based on the above analysis, the patient samples had between 13% and 20% of frataxin levels compared to the control.

** Cell culture samples derived from control individuals and FA patients were purchased from the Coriell Cell Repository (Camden, NJ).*

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TROUBLESHOOTING GUIDE

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.

Positive control band does not appear

Make sure that the wicking paper is in contact with the nitrocellulose membrane. Care should always be taken when handling the dipsticks to prevent disruption of this junction (see Figure 1).