ab201121 – Frataxin Human SimpleStep ELISA® Kit

Instructions for Use

For the quantitative measurement of Frataxin in human cell and tissue extracts.

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

Version 4 Last Updated 13 April 2022

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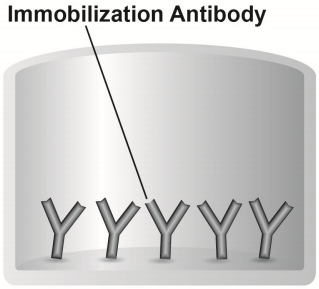
1. BACKGROUND

Frataxin *in vitro* SimpleStep ELISA® (Enzyme-Linked Immunosorbent Assay) kit is designed for the quantitative measurement of Frataxin protein in in human cell and tissue extracts.

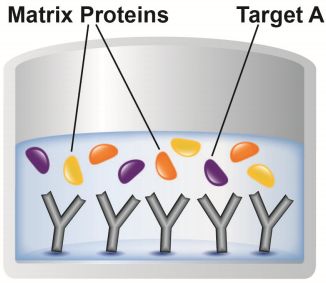
The SimpleStep ELISA® employs an affinity tag labeled capture antibody and a reporter conjugated detector antibody which immunocapture the sample analyte in solution. This entire complex (capture antibody/analyte/detector antibody) is in turn immobilized via immunoaffinity of an anti-tag antibody coating the well. To perform the assay, samples or standards are added to the wells, followed by the antibody mix. After incubation, the wells are washed to remove unbound material. TMB Development Solution is added and during incubation is catalyzed by HRP, generating blue coloration. This reaction is then stopped by addition of Stop Solution completing any color change from blue to yellow. Signal is generated proportionally to the amount of bound analyte and the intensity is measured at 450 nm. Optionally, instead of the endpoint reading, development of TMB can be recorded kinetically at 600 nm.

Frataxin is a 17 kDa nuclear-encoded mitochondrial protein. In humans the gene is localized on chromosome 9 and is highly conserved during evolution. The gene is expressed in every cell, although in varying levels in different tissues and during development. The specific function of frataxin is still unknown, but it has been shown to play a role in iron metabolism. Studies have demonstrated that the deletion of the frataxin gene in yeast results in iron accumulation in mitochondria and loss of respiration. Recombinant human frataxin has been shown to bind iron in vitro and increased mitochondrial iron levels have been observed in patients with Friedreich's Ataxia (FRDA). FRDA is an autosomal recessive, progressive degenerative disease characterized by neurodegeneration and cardiomyopathy it is the most common inherited ataxia.

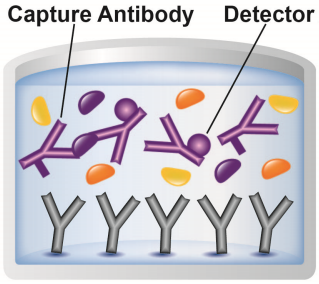
2. ASSAY SUMMARY



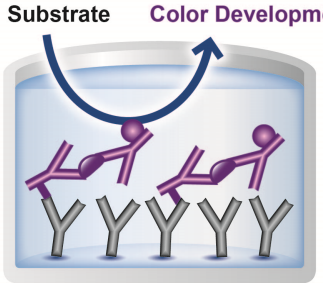
Remove appropriate number of antibody coated well strips. Equilibrate all reagents to room temperature. Prepare all reagents, samples, and standards as instructed.



Add standard or sample to appropriate wells.



Add Antibody Cocktail to all wells. Incubate at room temperature.



Aspirate and wash each well. Add TMB Development Solution to each well and incubate. Add Stop Solution at a defined endpoint.
Alternatively, record color development kinetically after TMB substrate addition.

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 2-8ºC immediately upon receipt apart from the Frataxin protein, which should be stored at -80ºC.

Refer to list of materials supplied for storage conditions of individual components. Observe the storage conditions for individual prepared components in sections 9 & 10.

5. MATERIALS SUPPLIED

Item	Amount	Storage Condition (Before Preparation)
10X Human Frataxin Capture Antibody	6 mL	+2-8ºC
10X Human Frataxin Detector Antibody	6 mL	+2-8ºC
Human Frataxin Recombinant Protein*	2 mL	-80ºC
Antibody Diluent 4BI	60 mL	+2-8ºC
5X Cell Extraction Buffer PTR	50 mL	+2-8ºC
50X Cell Extraction Enhancer	5 mL	+2-8ºC
10X Wash Buffer PT	200 mL	+2-8ºC
TMB Development Solution	120 mL	+2-8ºC
Stop Solution	120 mL	+2-8ºC
Sample Diluent NS	120 mL	+2-8ºC
Pre-Coated 96 Well Microplate (12 x 8 well strips)	10 x 96 Wells	+2-8ºC
Plate Seal	10	+2-8ºC

*It is recommended to aliquot the protein into single use vials, to prevent multiply freeze thaw cycles, which could affect the stability of the protein.

6. MATERIALS REQUIRED, NOT SUPPLIED

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

- Microplate reader capable of measuring absorbance at 450 or 600 nm.
- Method for determining protein concentration (BCA assay recommended).
- PBS (1.4 mM KH2PO4, 8 mM Na2HPO4, 140 mM NaCl, 2.7 mM KCl, pH 7.4).
- Deionized water.
- Multi- and single-channel pipettes.
- Tubes for standard dilution.
- Plate shaker for all incubation steps.
- 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

- Samples generating values higher than the highest standard should be further diluted in the appropriate sample dilution buffers.
- 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.
- Ensure plates are properly sealed or covered during incubation steps.
- Complete removal of all solutions and buffers during wash steps is necessary to minimize background.
- As a guide, typical ranges of sample concentration for commonly used sample types are shown below in Sample Preparation (section 11).
- All samples should be mixed thoroughly and gently.
- Avoid multiple freeze/thaw of samples.
- Incubate ELISA plates on a plate shaker during all incubation steps.
- When generating positive control samples, it is advisable to change pipette tips after each step.
- **This kit is sold based on number of tests. A 'test' simply refers to a single assay well. The number of wells that contain sample, control or standard will vary by product. Review the protocol completely to confirm this kit meets your requirements. Please contact our Technical Support staff with any questions.**

9. REAGENT PREPARATION

- Maintain bulk reagents at 4°C and remove the volume required for the day of experiment. Equilibrate this material to room temperature (18-25°C) prior to use. **The sample volumes below are sufficient for 96 wells (12 x 8-well strips); adjust volumes as needed for the number of strips in your experiment.**
- Prepare only as much reagent as is needed on the day of the experiment. Capture and Detector Antibodies have only been tested for stability in the provided 10X formulations.

9.1 1X Cell Extraction Buffer PTR

Prepare 1X Cell Extraction Buffer PTR by diluting 5X Cell Extraction Buffer PTR and 50X Cell Extraction Enhancer Solution to 1X with deionized water. To make 10 mL 1X Cell Extraction Buffer PTR combine 7.8 mL deionized water, 2 mL 5X Cell Extraction Buffer PTR and 200 µL 50X Cell Extraction Enhancer Solution Mix thoroughly and gently. If required protease inhibitors can be added.

Alternative – Enhancer may be added to 1X Cell Extraction Buffer PTR after extraction of cells or tissue. Refer to note in the Troubleshooting section.

9.2 1X Wash Buffer PT

Prepare 1X Wash Buffer PT by diluting 10X Wash Buffer PT with deionized water. To make 100 mL 1X Wash Buffer PT combine 10 mL 10X Wash Buffer PT with 90 mL deionized water. Mix thoroughly and gently.

9.3 Antibody Cocktail

Prepare Antibody Cocktail by diluting the capture and detector antibodies in Antibody Diluent 4BI. To make 6 mL of the Antibody Cocktail combine 600 µL 10X Capture Antibody and 600 µL 10X Detector Antibody with 4.8 mL Antibody Diluent 4BI. Mix thoroughly and gently.

10. STANDARD PREPARATION

Prepare serially diluted standards immediately prior to use. Always prepare a fresh set of positive controls for every use.

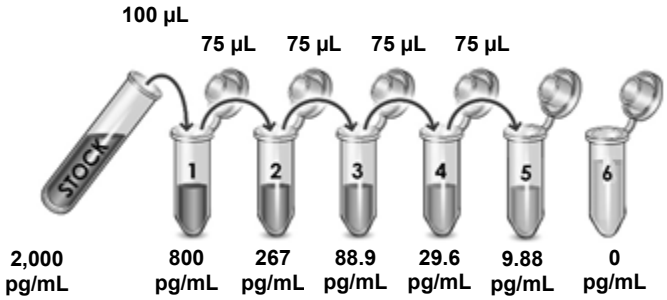
The following table describes the preparation of a standard curve for duplicate measurements (recommended).

It is recommended to aliquot and store the provided protein standard into single use vials to prevent multiply freeze/thaw cycles.

10.1 **IMPORTANT:** If the protein standard vial has a volume identified on the label, dilute the Frataxin standard by adding that volume of 1X Cell Extraction Buffer PTR indicated on the label. Alternatively, if the vial has a mass & concentration identified, thaw the Frataxin standard on ice. Hold at room temperature for 10 minutes and mix gently. This is the 2,000 pg/mL **Stock Standard** Solution.

10.2 Label six tubes, Standards 1–6 and add 150 µL appropriate 1X Cell Extraction Buffer PTR into each tube.

10.3 Use the Stock Standard to prepare the following dilution series. Standard #6 contains no protein and is the Blank control:



11. SAMPLE PREPARATION

TYPICAL SAMPLE DYNAMIC RANGE	
Sample Type	**Range (µg/mL)**
HeLa Cell Extract	0.5 – 100
A431 Cell Extract	0.5 – 100
143B Cell Extract	0.5 – 300
SH-SY5Y Cell Extract	0.1 – 50
HL60 Cell Extract	0.1 – 50
MCF7 Cell Extract	0.1 – 50
HEK293 Cell Extract	0.05 – 30
HepG2 Cell Extract	< 10

11.1 Preparation of extracts from cell pellets

11.1.1 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 4ºC.

11.1.2 Rinse cells twice with PBS.

11.1.3 Solubilize pellet at $2x10^7$ cell/mL in chilled extraction buffer.

11.1.4 Incubate on ice for 20 minutes.

11.1.5 Centrifuge at 18,000 x g for 20 minutes at 4°C.

11.1.6 Transfer the supernatants into clean tubes and discard the pellets.

11.1.7 Assay samples immediately or aliquot and store at -80°C. The sample protein concentration in the extract may be quantified using a protein assay.

11.1.8 Dilute samples to desired concentration in extraction buffer.

11.2 **Preparation of extracts from adherent cells by direct lysis (alternative protocol)**

11.2.1 Remove growth media and rinse adherent cells 2 times in PBS.

11.2.2 Solubilize the cells by addition of chilled extraction buffer directly to the plate (use 750 µL - 1.5 mL extraction buffer per confluent 15 cm diameter plate).

11.2.3 Scrape the cells into a microfuge tube and incubate the lysate on ice for 15 minutes.

11.2.4 Centrifuge at 18,000 x g for 20 minutes at 4°C.

11.2.5 Transfer the supernatants into clean tubes and discard the pellets.

11.2.6 Assay samples immediately or aliquot and store at -80°C. The sample protein concentration in the extract may be quantified using a protein assay.

11.2.7 Dilute samples to desired concentration in extraction buffer.

11.3 **Preparation of extracts from tissue homogenates**

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

11.3.2 Homogenize 100 to 200 mg of wet tissue in 500 µL - 1 mL of chilled extraction buffer. For lower amounts of tissue adjust volumes accordingly.

11.3.3 Incubate on ice for 20 minutes.

11.3.4 Centrifuge at 18,000 x g for 20 minutes at 4°C.

11.3.5 Transfer the supernatants into clean tubes and discard the pellets.

11.3.6 Assay samples immediately or aliquot and store at -80°C. The sample protein concentration in the extract may be quantified using a protein assay.

11.3.7 Dilute samples to desired concentration in extraction buffer.

12. PLATE PREPARATION

- The 96 well plate strips included with this kit are supplied ready to use. It is not necessary to rinse the plate prior to adding reagents.
- Unused plate strips should be immediately returned to the foil pouch containing the desiccant pack, resealed and stored at 4°C.
- For each assay performed, a minimum of two wells must be used as the zero control.
- For statistical reasons, we recommend each sample should be assayed with a minimum of two replicates (duplicates).
- Differences in well absorbance or “edge effects” have not been observed with this assay.

13. ASSAY PROCEDURE

- **Maintain stock reagents at 4°C and only equilibrate to room temperature the volume of reagent necessary.**
- **It is recommended to assay all standards, controls and samples in duplicate.**

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

13.2 Remove excess microplate strips from the plate frame, return them to the foil pouch containing the desiccant pack, reseal and return to 4ºC storage.

13.3 Add 50 µL of each sample or standards to appropriate wells.

13.4 Add 50 µL of the Antibody Cocktail to each well.

13.5 Seal the plate and incubate for 1 hour at room temperature on a plate shaker set to 400 rpm.

13.6 Wash each well with 3 x 350 µL 1X Wash Buffer PT. Wash by aspirating or decanting from wells then dispensing 350 µL 1X Wash Buffer PT into each well. Complete removal of liquid at each step is essential for good performance. After the last wash invert the plate and blot it against clean paper towels to remove excess liquid.

13.7 Add 100 µL of TMB Development Solution to each well and incubate for 10 minutes in the dark on a plate shaker set to 400 rpm.

Given variability in laboratory environmental conditions, optimal incubation time may vary between 5 and 20 minutes.

Note: The addition of Stop Solution will change the color from blue to yellow and enhance the signal intensity about 3X. To avoid signal saturation, proceed to the next step before the high concentration of the standard reaches a blue color of O.D.600 equal to 1.0.

13.8 Add 100 µL of Stop Solution to each well. Shake plate on a plate shaker for 1 minute to mix. Record the OD at 450 nm. This is an endpoint reading.

Alternative to 13.7 – 13.8: Instead of the endpoint reading at 450 nm, record the development of TMB Substrate kinetically. Immediately after addition of TMB Development Solution begin recording the blue color development with elapsed time in the microplate reader prepared with the following settings:

Mode:	Kinetic
Wavelength:	600 nm
Time:	up to 20 min
Interval:	20 sec - 1 min
Shaking:	Shake between readings

Note that an endpoint reading can also be recorded at the completion of the kinetic read by adding 100 µL Stop Solution to each well and recording the OD at 450 nm.

13.9 Analyze the data as described below.

14. CALCULATIONS

14.1 Calculate the average absorbance value for the blank control (zero) standards. Subtract the average blank control standard absorbance value from all other absorbance values.

14.2 Create a standard curve by plotting the average blank control subtracted absorbance value for each standard concentration (y-axis) against the target protein concentration (x-axis) of the standard. Use graphing software to draw the best smooth curve through these points to construct the standard curve.

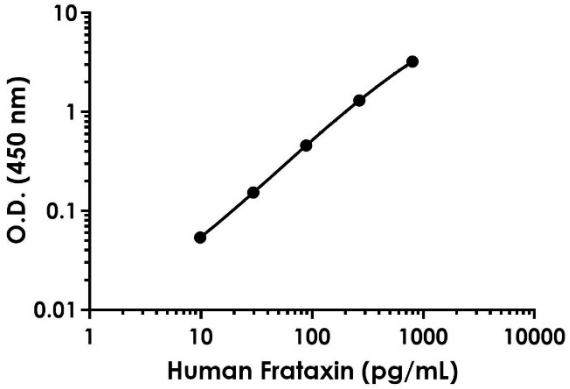
Note: Most microplate reader software or graphing software will plot these values and fit a curve to the data. A four parameter curve fit (4PL) is often the best choice; however, other algorithms (e.g. linear, semi-log, log/log, 4 parameter logistic) can also be tested to determine if it provides a better curve fit to the standard values.

14.3 Determine the concentration of the target protein in the sample by interpolating the blank control subtracted absorbance values against the standard curve. Multiply the resulting value by the appropriate sample dilution factor, if used, to obtain the concentration of target protein in the sample.

14.4 Samples generating absorbance values greater than that of the highest standard should be further diluted and reanalyzed. Similarly, samples which measure at an absorbance values less than that of the lowest standard should be retested in a less dilute form.

15. TYPICAL DATA

TYPICAL STANDARD CURVE – Data provided for **demonstration purposes only**. A new standard curve must be generated for each assay performed.



Standard Curve Measurements			
Conc. (pg/mL)	O.D. 450 nm		Mean O.D.
	1	2	
0	0.056	0.063	0.060
9.88	0.117	0.111	0.114
29.6	0.223	0.204	0.213
88.9	0.552	0.486	0.519
267	1.432	1.309	1.371
800	3.368	3.207	3.288

Figure 1. Example of Frataxin standard curve. The Frataxin standard curve was prepared as described in Section 10. Raw data values are shown in the table. Background-subtracted data values (mean +/- SD) are graphed.

16. TYPICAL SAMPLE VALUES

SENSITIVITY –

The calculated minimal detectable (MDD) dose is 2.1 pg/mL. The MDD was determined by calculating the mean of zero standard replicates (n=34) and adding 2 standard deviations then extrapolating the corresponding concentrations.

RECOVERY –

(Sample spiking in representative sample matrices)

Sample Type	Range (%)	Average % Recovery
50% Cell Culture Media	93 - 101	97
10% Human Serum	86 - 98	92
10% Human Plasma - EDTA	88 - 92	89

LINEARITY OF DILUTION –

Linearity of dilution is determined based on interpolated values from the standard curve. Linearity of dilution defines a sample concentration interval in which interpolated target concentrations are directly proportional to sample dilution.

Dilution Factor	Interpolated value	Sample Diluent NS	Cell Culture Media	Human Serum	Human Plasma (EDTA)
Undiluted	pg/mL	191.32	181.03	166.62	172.75
	% Expected Value	**100**	**100**	**100**	**100**
1:2	pg/mL	98.97	93.05	84.59	81.66
	% Expected Value	**103**	**103**	**102**	**95**
1:4	pg/mL	47.36	46.92	44.03	45.23
	% Expected Value	**99**	**104**	**106**	**105**
1:8	pg/mL	23.48	23.12	20.58	20.98
	% Expected Value	**98**	**102**	**99**	**97**
1:16	pg/mL	10.19	10.35	9.21	10.48
	% Expected Value	**85**	**91**	**88**	**97**

PRECISION –

Mean coefficient of variations of interpolated values from 3 concentrations of HeLa extracts within the working range of the assay.

	Intra-Assay	Inter-Assay
n=	9	3
CV (%)	2.3	6.3

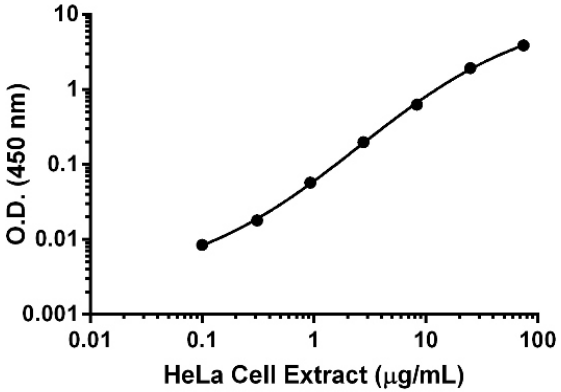


Figure 2. Titration of HeLa cell extract within the working range of the assay. Background subtracted data from duplicate measurements are plotted.

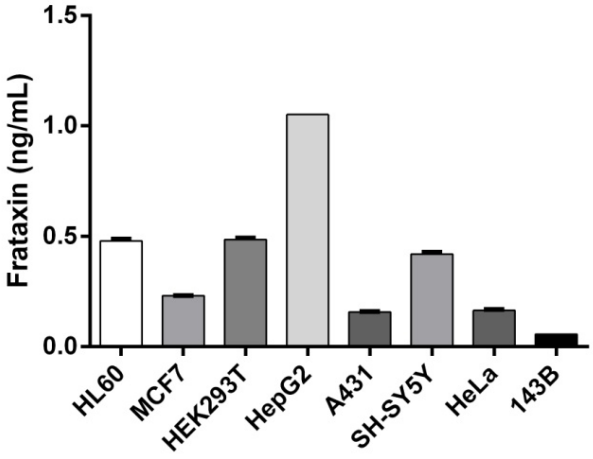


Figure 3. Quantification of Frataxin expression in different cell lines. Interpolated values of Frataxin are plotted for the indicated cell lines based on an extract load of 10 µg/mL.

17. ASSAY SPECIFICITY

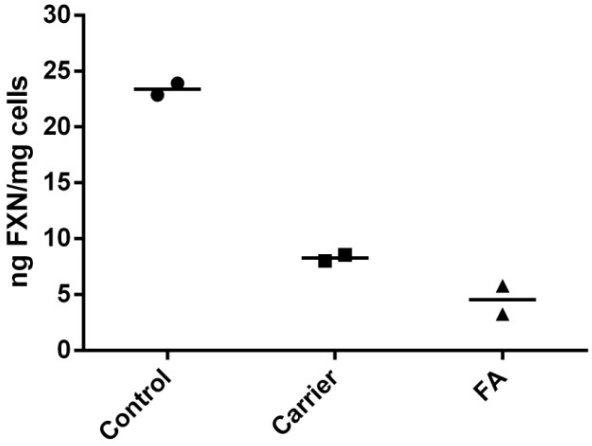


Figure 4. Quantification of Frataxin expression in different human patient samples. Transformed B lymphocyte cells from Friedreich's Ataxia (FRDA) samples, due to a stable homozygous GAA repeat insertion, are compared to heterozygous carrier B lymphocyte cells and control B lymphocyte cells. B lymphocyte cell extracts were analyzed across a 7-point titration (0.1-100 µg/mL) and frataxin levels were interpolated from the standard curve. Average interpolated values of Frataxin are plotted.

18. SPECIES REACTIVITY

This kit detects Frataxin in human cell culture and tissue extract samples only.

Serum, plasma, saliva, and urine samples have not been tested with this kit.

Please contact our Technical Support team for more information.

19. TROUBLESHOOTING

Problem	Cause	Solution
Poor standard curve	Inaccurate Pipetting	Check pipettes
	Improper standard dilution	Prior to opening, briefly spin the stock standard tube and dissolve the powder thoroughly by gentle mixing
Low Signal	Incubation times too brief	Ensure sufficient incubation times; increase to 2 or 3 hour standard/sample incubation
	Inadequate reagent volumes or improper dilution	Check pipettes and ensure correct preparation
	Incubation times with TMB too brief	Ensure sufficient incubation time until blue color develops prior addition of Stop solution
Large CV	Plate is insufficiently washed	Review manual for proper wash technique. If using a plate washer, check all ports for obstructions.
	Contaminated wash buffer	Prepare fresh wash buffer
Low sensitivity	Improper storage of the ELISA kit	Store your reconstituted standards at -80°C, all other assay components 4°C. Keep TMB Development Solution protected from light.
Precipitate in Diluent	Precipitation and/or coagulation of components within the Diluent.	Precipitate can be removed by gently warming the Diluent to 37ºC.

20. **NOTES**

Technical Support

For all technical or commercial enquiries please go to:

www.abcam.com/contactus
www.abcam.cn/contactus (China)
www.abcam.co.jp/contactus (Japan)