abcam

Product datasheet

Human Frataxin ELISA Kit ab176112

SimpleStep ELISA

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Overview

Product name Human Frataxin ELISA Kit

Detection methodColorimetric

Precision Intra-assay

Sample	n	Mean	SD	CV%
HeLa	9			2.3%

Inter-assay

Sample	n	Mean	SD	CV%
HeLa	3			6.3%

Sample type Cell culture extracts, Tissue Extracts

Assay type Sandwich (quantitative)

Sensitivity 2.1 pg/ml

Range 9.88 pg/ml - 800 pg/ml

Recovery Sample specific recovery

Sample type	Average %	Range
Serum	92	86% - 98%
Cell culture media	97	93% - 101%
EDTA Plasma	89	88% - 92%

Assay time 1h 30m

Assay duration One step assay

Species reactivity Reacts with: Human

Product overview Human Frataxin ELISA kit (ab176112) is a single-wash 90 min sandwich ELISA designed for the

quantitative measurement of Frataxin protein in human cell culture and tissue extracts. It uses our

proprietary SimpleStep ELISA® technology. Quantitate human Frataxin with 1.1 pg/ml sensitivity.

SimpleStep ELISA® technology employs capture antibodies conjugated to an affinity tag that is recognized by the monoclonal antibody used to coat our SimpleStep ELISA® plates. This approach to sandwich ELISA allows the formation of the antibody-analyte sandwich complex in a single step, significantly reducing assay time. See the SimpleStep ELISA® protocol summary in the image section for further details. Our SimpleStep ELISA® technology provides several benefits:

- -Single-wash protocol reduces assay time to 90 minutes or less
- -High sensitivity, specificity and reproducibility from superior antibodies
- -Fully validated in biological samples
- -96-wells plate breakable into 12 x 8 wells strips

A 384-well SimpleStep ELISA® microplate (ab203359) is available to use as an alternative to the 96-well microplate provided with SimpeStep ELISA® kits.

ASSAY SPECIFICITY This kit recognizes both native and recombinant human Frataxin protein in cell and tissue extract samples only. Serum, plasma, saliva, and urine samples have not been tested with this kit.

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.

Abcam has not and does not intend to apply for the REACH Authorisation of customers' uses of products that contain European Authorisation list (Annex XIV) substances. It is the responsibility of our customers to check the necessity of application of REACH Authorisation, and any other relevant authorisations, for their intended uses.

Platform

Microplate

Properties

Storage instructions

Store at +4°C. Please refer to protocols.

Components	1 x 96 tests
10X Human Frataxin Capture Antibody	1 x 600µl
10X Human Frataxin Detector Antibody	1 x 600µl
10X Wash Buffer PT (ab206977)	1 x 20ml
50X Cell Extraction Enhancer Solution (ab193971)	1 x 1ml

Notes

Components	1 x 96 tests
5X Cell Extraction Buffer PTR (ab193970)	1 x 10ml
Antibody Diluent 4BI	1 x 6ml
Human Frataxin Lyophilized Recombinant Protein	2 vials
Plate Seals	1 unit
Sample Diluent NS (ab193972)	1 x 12ml
SimpleStep Pre-Coated 96-Well Microplate (ab206978)	1 unit
Stop Solution	1 x 12ml
TMB Development Solution	1 x 12ml

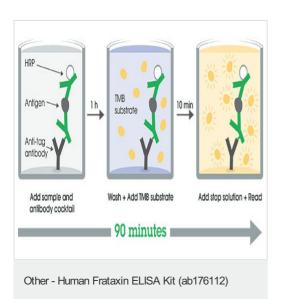
Relevance

Function: Promotes the biosynthesis of heme and assembly and repair of iron-sulfur clusters by delivering Fe2+ to proteins involved in these pathways. May play a role in the protection against iron-catalyzed oxidative stress through its ability to catalyze the oxidation of Fe2+ to Fe3+; the oligomeric form but not the monomeric form has in vitro ferroxidase activity. May be able to store large amounts of iron in the form of a ferrihydrite mineral by oligomerization; however, the physiological relevance is unsure as reports are conflicting and the function has only been shown using heterologous overexpression systems. Modulates the RNA-binding activity of ACO1.

Cellular localization

Cytoplasmic and Mitochondrial

Images

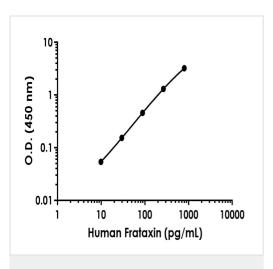


SimpleStep ELISA technology allows the formation of the antibodyantigen complex in one single step, reducing assay time to 90 minutes. Add samples or standards and antibody mix to wells all at once, incubate, wash, and add your final substrate. See protocol for a detailed step-by-step guide.

Standard Curve Measurement				
Concentration (pg/mL)	O.D 4	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	

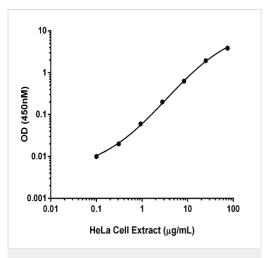
Raw data values are shown in the table

Raw data value for example of frataxin standard curve

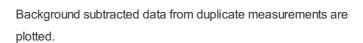


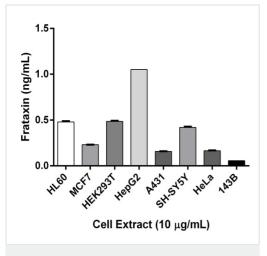
Background-subtracted data values (mean +/- SD) are graphed.

Example of Frataxin standard curve



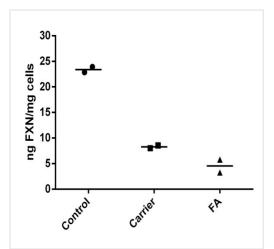
Titration of HeLa cell extract within the working range of the assay





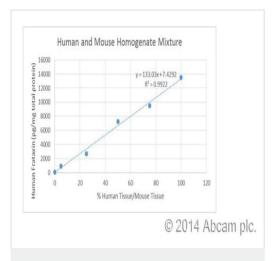
Quantification of Frataxin expression in different cell lines

Interpolated values of Frataxin are plotted for the indicated cell lines based on a extract load of 10 μ g/mL.



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 $\mu g/mL)$ and frataxin levels were interpolated from the standard curve. Average interpolated values of Frataxin are plotted.



Standard curve generated using Abcam's Frataxin Human SimpleStep ELISA™ Kit (ab176112) with human brain tissue homogenate mixed in different proportions with mouse brain tissue homogenate. ab176112 did not cross-react with mouse frataxin. Both kinetic and endpoint readouts were used sequentially for a larger dynamic range. ab110353, 12ng human frataxin full length protein was also used to extended the standard curve to 12ng/ml.

Sandwich ELISA - Frataxin Human SimpleStep ELISA TM Kit (ab176112)

This image is courtesy of an anonymous Abreview

Dilution Factor	Interpolated value	Sample Diluent	50% Conditioned Media	10% Human Serum	10 % Human Plasma (EDTA)
Undiluted-	pg/mL	191.32	181.03	166.62	172.75
	% Expected value	100	100	100	100
2	pg/mL	98.97	93.15	84.59	81.66
	% Expected value	103	103	102	95
4	pg/mL	47.36	46.92	44.03	45.23
	% Expected value	99	104	106	105
8	pg/mL	23.48	23.12	20.58	20.98
	% Expected value	98	102	99	97
1/	pg/mL	10.19	10.35	9.21	10.48
16	% Expected value	85	91	88	97

Recombinant Frataxin was measured in biological samples in a 2-fold dilution series

Linearity of dilution for Recombinant Frataxin

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