

Assays to measure frataxin protein levels

Comparison of Types of Relatively High-Throughput Frataxin Assays reported in the literature.

Friedreich's Ataxia is caused by low levels of the protein frataxin, a consequence of mutations in the frataxin gene. Many potential therapies aim to increase levels of frataxin, targeting the root cause of the disease. Thus, sensitive, accurate and quantitative measurement of frataxin is an important biomarker in drug development, while understanding protein levels is also important in basic research. This document lists published immunoassays for the detection of frataxin in cell culture and in tissues with their characteristics and antibodies used. A list of antibodies used for low throughput assays, like western blotting, is provided at the end of the document. Please contact Liz Soragni (liz.soragni@curefa.org) if you would like an introduction to specific investigators who have developed or validated methods or would like more information.

	Dipstick	Luminex	ELISA	ECLIA	Liquid Chromatography-High Resolution Mass Spectrometry
Method	Willis et al, 2008 and Deutsch et al, 2010, Plasterer et al., 2013	Oglesbee et al, 2013	Plasterer et al, 2013, Koeppen et al. 2011, Boehm et al. 2011	Steinkellner et al, 2010	Guo et al., 2018 a and b
Detection	Rapid, noninvasive lateral-flow immunoassay. Anti-frataxin mAbs were generated by immunizing mice (F ₁ BALB/cJ × SLJ/J) with soluble, native recombinant human frataxin; amino acids 56–210 (Cavadini, et al. Hum Mol Genet. 2002;11:217). This construct corresponds to the 155 amino acid form of frataxin that was thought to be the mitochondrial form after removal of the mitochondrial targeting sequence by mitochondrial processing peptidase (MPP). The two mAbs	Antibody pairs were used to capture FXN and an internal control protein, ceruloplasmin. Polyclonal, anti-FXN, rabbit detector antibodies (PAC 2517), and purified recombinant human FXN isoforms (FXN81–210 and FXN56–210) were generated and characterized as previously reported (Gakh et al. J Biol Chem. 2010;285:38486). Additional reagents were purchased from MitoSciences (no	Enzyme-linked immunosorbent assay. The assay system utilizes a mouse anti-frataxin antibody for the solid-phase (microtiter wells) immobilization. A rabbit anti-frataxin antibody was used as secondary antibody. Frataxin levels were normalized to the protein content in the samples. The protein content of the samples was determined by a Bradford protein assay (Bio-Rad, Richmond, CA).	Electrochemiluminescence assay (ECLIA) to measure frataxin protein levels. The primary mouse anti-frataxin monoclonal capture antibody was purchased from Chemicon (Clone: 1G2, #MAB1594) (Millipore); the secondary frataxin (H-155) rabbit polyclonal antibody was from Santa Cruz Biotechnology. The third antibody which was used as	The assay is based on stable isotope dilution immunopurification two-dimensional nano-ultrahigh performance liquid chromatography/parallel reaction monitoring/mass spectrometry.

	selected as dipstick immunocapture (clone ID# 17A11AC7) and detector (clone ID# 18A5DB1) mAbs recognize frataxin in all assay formats (Western, immunofluorescence, dipstick, ELISA etc.).	longer available), including monoclonal, anti-FXN, mouse capture antibody (Anti-Frataxin antibody [17A11]), and recombinant human FXN protein. Polyclonal anti-CP rabbit antibodies were purchased from Cortex Biochem.		detection antibody was goat anti-rabbit HRP-Sulfo-TAG™ from Meso Scale Discovery (Gaithersburg, US).	
Uses	Diagnostic, FXN measurement in peripheral tissues, biomarkers, assessment of disease severity	Under validation for newborn screening, high throughput screening, available as a diagnostic test to be used as an adjunct to genetic testing			Can detect size of frataxin and absolute amounts.
Calibration	The relationship between frataxin loaded and dipstick signal was linear through 500 pg of recombinant frataxin and did not saturate until approximately 4,000 pg. The assay's linear range could be expanded significantly by normalizing the frataxin signal to the signal of the "procedural control" (goat anti-mouse IgG, GAM) band, which is an internal positive control run on each dipstick	Reference adult and pediatric FXN concentrations ranged from 15 to 82 ng/mL (median, 33 ng/mL) for dried blood and whole blood.		Recombinant frataxin can be measured over a wide range (0–2000 picograms (pg) per well, with $R^2 = 0.99$)	Calibration standards were prepared by spiking appropriate amounts of the frataxin standard to 500 µg frataxin-depleted platelet lysates or 500 µg of BSA to make the final concentrations of 0.078, 0.156, 0.313, 0.625, 1.25, 2.5, 5, 10, 20, 40 pg/µg protein. The lower limit of quantification (LLOQ) of 0.08 pg/µg protein was defined as the lowest QC sample.
Reliability	Intra-assay (3.3–11.2% and 0.5–	Intraassay (CV) values were 4.9%–	CV was 15.6% over a period of several	Intraassay: 1.4%–3.8%	100% sensitivity and specificity for

	12.2%, control and patient, respectively), interassay (10.3% and 5.3%, control and patient, respectively), and intersample (17.4%, control) coefficient of variation (CV) values.	13%. Interassay imprecision (CV) values were 9.8%–16%,	weeks (intersample)	depending on tissue, Interassay: 1.8–7.2% depending on tissue	discriminating between controls and FA cases. Variance: intraday: (n=5) precision 5.1%, accuracy 96.8%; interday (n=5), precision 3.7%, accuracy 101.0%
Sensitivity	Accurately measures picogram levels of frataxin protein - 40 and 4000 pg/test or approximately 0.1 – 10 nM of sample.	The FXN limit of detection was 0.07 ng/mL, and the reportable range of concentrations was 2–200 ng/mL.		Pictograms, narrow range can be measured.	The lower limit of quantification is 0.08 pg frataxin/μg protein
Tissues that can be assayed	Buccal cells, Whole blood and lymphoblastoid lines have been tested.	Whole blood, dried blood spots		Multiple tissues, require invasive collection of tissues	Platelets, whole blood, likely other tissues. Can distinguish between FXN from human and other species (mouse and non-human primates)
Notes	Western blot examination of lymphoblast whole cell extracts showed significant amounts of the intermediate and mature forms of frataxin (frataxin42–210 and frataxin56–210), while only trace amounts of the precursor were revealed.	Very high throughput	Can be done in 96 well plate format, human or mouse	Labor intensive, despite 96 well format.	Quantitative.

Frataxin ELISA Kits

FARA is grateful to the companies and academic groups that have developed systems for measurement of human and mouse frataxin and to the research community for characterization of these assays, which are mostly ELISA assays.

An overview of currently available human and mouse assay kits available commercially or referenced in the literature is detailed below:

Commercial Human Frataxin ELISA Kits				
Frataxin Assay	Vendor	Catalogue #	References	Product Link
ELISA	Abcam	ab176112	3	http://www.abcam.com/human-frataxin-elisa-kit-ab176112.html
Dipstick ELISA	Abcam	ab109881	16	http://www.abcam.com/frataxin-protein-quantity-dipstick-assay-kit-ab109881.html*
ELISA	Abcam	ab201121	0	http://www.abcam.com/human-frataxin-elisa-kit-10-x-96-well-plate-ab201121.html
ELISA	Millipore	EAMT002 (Discontinued)	0	http://www.emdmillipore.com/US/en/product/Frataxin-ELISA-Kit,EMD_BIO-EAMT002
ELISA	Biomatik	EKC33754	0	http://www.biomatik.com/products/elisa-kits/human-frataxin-mitochondrial-fxn-elisa-kit.html
ELISA	Antibodies online	ABIN4993459	0	https://www.antibodies-online.com/kit/4993459/Frataxin+FXN+ELISA+Kit/?utm_source=partner&utm_medium=biocompare&utm_campaign=non_sponsored&utm_content=kit&utm_term=ABIN4993459
ELISA	Antibodies online	ABIN2636770	0	https://www.antibodies-online.com/kit/2636770/Frataxin+FXN+ELISA+Kit/?utm_source=partner&utm_medium=biocompare&utm_campaign=non_sponsored&utm_content=kit&utm_term=ABIN2636770
ELISA	Aviva Systems Biology	OKCA01248	0	https://www.avivasysbio.com/en/fxn-elisa-kit-human-okca01248.html
ELISA	LifeSpan Biosciences	LS-F23370	0	https://www.lsbio.com/elisakits/human-fxn-frataxin-elisa-kit-sandwich-elisa-ls-f23370/23370?trid=247
ELISA	MyBioSource	MBS935917	1	https://www.mybiosource.com/prods/ELISA-Kit/Human/frataxin/FXN/datasheet.php?products_id=935917#QLREF

ELISA	MyBioSource	MBS2515805	1	https://www.mybiosource.com/prods/ELISA-Kit/Human/frataxin/FXN/datasheet.php?products_id=2515805#QLREF
ELISA	Biorbyt	orb404450	0	http://www.biorbyt.com/human-fxn-elisa-kit
AlphaLISA	PerkinElmer	AL322HV (100 assay); AL322C (500 assay); AL322F (5,000 assay)	0	http://www.perkinelmer.com/product/alphalisa-hfrataxin-kit-100pts-al322hv

*Check online comments on this kit.

Frataxin antibodies for western blotting analysis

The table below is adapted from Weng et al. J Immunol Methods. 474:112629

(<https://www.sciencedirect.com/science/article/pii/S0022175919301097>). All antibodies listed, except for Millipore MABN2313, are compared in the Weng et al. paper for detection in human and animal samples by western blotting.

Supplier	Catalog number	Species	Clone	Epitope reported
Abcam	Ab113691	Mouse	17A11	Full length protein
Abcam	Ab175402	Rabbit	Polyclonal	Full length protein
Millipore-Sigma	MAB1594	Mouse	1G2	Full length fused to TrpE
LifeSpan Bio	LS-C197243	Mouse	1D9	Synthetic peptide (aa91–200) with GST tag
Abcam	Ab124680	Rabbit	EPR6107	Synthetic peptide (aa 150 – C-terminus
Millipore	MABN2313 [Developed in the Puccio lab at IGBMC and commercially available since 2020. Detects mature and intermediate forms of human Frataxin]	Mouse	4F9	Full-length human recombinant Frataxin

Antibody performance in western blotting (Weng et al. J Immunol Methods. 474:112629)

Catalog number	His-tag monkey frataxin	His-tag human frataxin	His-tag mouse mature frataxin	His-tag mouse intermediate frataxin	Mouse Tissue		
					Endog mature frataxin	Immuno-reactive protein (22.4 kDa)	His-tag mouse frataxin
Ab113691	None	Strong	Weak	Strong	None	Strong	None
Ab175402	Strong	Strong	Strong	Strong	Strong	None	Strong
MAB1594	Weak	Weak	Weak	Strong	Weak	Strong	Weak
LS-C197243	Strong	Strong	None	Strong	None	Strong	None
Ab124680	None	None	None	Very weak	None	Interference	None