NULISAqpcr™ NfL (NEFL) Assay

DATA SHEET

Introduction

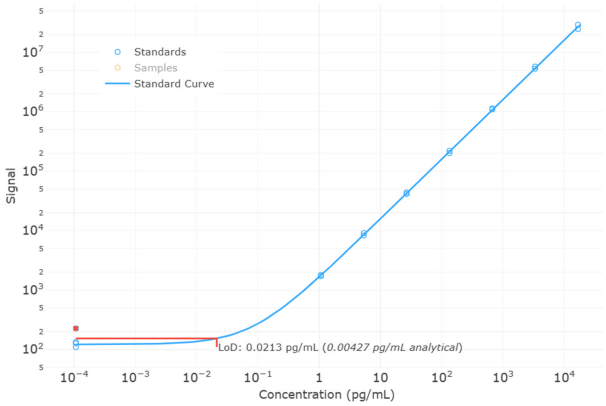
Neurofilament light (NfL, NEFL) is expressed in neuronal cytoplasm. It serves as a structural protein within neurons, providing mechanical support to axons, facilitating axonal transport, and potentially modulating neuronal signaling. NfL is released into the extracellular space and subsequently into the CSF and blood following axonal damage. Elevated levels of NfL can indicate the extent of neuronal injury and are useful for diagnosing and monitoring the progression of neurological diseases, such as Amyotrophic Lateral Sclerosis (ALS), Alzheimer's Disease (AD), Multiple Sclerosis (MS), etc.

NULISA™ Technology

NULISA is a sandwich immunoassay in which two target specific antibodies, conjugated with unique oligonucleotide tags, bind and form a complex with the specific protein in solution. The resulting immunocomplex is purified in sequential capture and release steps to remove background and unbound antibodies. Successful formation of the immunocomplex brings the two oligonucleotides tags into proximity for a ligation reaction and the resulting product read out using quantitative PCR. The NULISAqpcr assay protocol is fully automated on the ARGO HT System which has an on-board qPCR reader and data is analyzed using the ARGO Command Center.

Preliminary Assay Performance

	Functional
LOD	0.029 pg/mL
LLoQ	1.081 pg/mL
ULoQ	17,000 pg/mL
Dynamic Range	1.081 – 17,000 pg/mL



The LoD (Limit of Detection) is the lowest concentration of the analyte that can be distinguished from the background signal of the assay and LoD for each run is calculated as 2.5 standard deviations above the average of the blanks. Assay LoD is the average of multiple runs.

The LLoQ (Lower Limit of Quantification) is the lowest concentration of the analyte that can be reliably quantified by the assay and defined as the lowest concentration measurable by the assay with CV ≤ 25% and 75-125% recovery.

The ULoQ (Upper limit of Quantitation) is the highest concentration of the analyte that can be reliably quantified by the assay and defined as the highest concentration measurable by the assay with CV ≤ 25% CV and75-125% recovery.

The Dynamic range is defined as the range of concentrations that an assay can reliably quantitate between the LLoQ to the ULoQ

Low Quality Controls (QCs) included in the target kits, consisting of lyophilized plasma with predetermined NfL concentrations. These QCs are treated as samples in the run to verify that the generated data consistently meet our accuracy and precision criteria.

Sample Controls

Internal and external controls are included in each run for data normalization and quality control purposes. Internal control is nucleic acid based and added to each well of the plate to monitor well to well variation and serves normalization purpose. External controls are High and Low Quality Controls (QCs) included in the target kits, consisting of lyophilized plasma with predetermined NfL concentrations. These QCs are treated as samples in the run to verify that the generated data consistently meet our accuracy and precision criteria.

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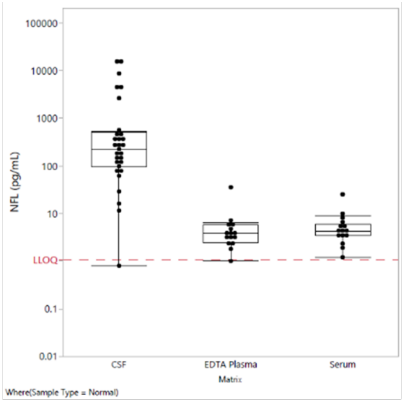
Sample Detectability & Reference Ranges

NfL was detected above LOD in healthy normal samples from EDTA plasma (n=15), serum (n=15) and CSF (n=29).

NfL was detected above LOD in all sample types tested.



NfL concentrations in CSF, EDTA Plasma, and serum from normal samples

Precision

Intra-assay precision or repeatability measures the variation in results obtained when a single sample is assayed multiple times within the same assay run. Inter-assay precision or reproducibility measures the variation in results obtained when the same sample is assayed multiple times in different assay runs or on different days. Precision was determined from a set of three independent runs.

Sample	Sample Category	Average Conc. (pg/mL)	Total %CV	Inter-assay %CV	Intra-assay %CV
PL-1	EDTA Plasma	6.43	10.79	2.68	10.46
PL-2		4.72	9.04	7.90	4.41
PL-3		3.02	18.73	12.88	13.60
PL-4		2.30	5.60	2.65	4.93
PL-5		4.76	14.24	13.79	3.52
PL-6		3.50	11.75	5.22	10.53
PL-7		1.97	12.89	12.60	2.75
PL-8		5.38	12.82	9.82	8.25

Parallelism

Parallelism confirms that the binding characteristic of the endogenous analyte to the antibodies is the same as for the calibrator. Each individual sample undergoes a serial dilution. Subsequently, the dilution series for each sample is assessed for recovery to ensure a consistent response across all samples.

Parallelism was assessed with 5 EDTA plasma samples serially diluted 2x with sample dilution buffer for up to 32x dilutions. Percent recovery was normalized to the dilution adjusted, neat concentration. All tested samples displayed parallelism with an average of 108% recovery across all dilution levels above the assay LLOQ.

Tested Sample	Avg Recovery	Recovery Range			Concentration Range (pg/mL)		
P1	107%	105%	-	109%	5	-	110
P2	110%	106%	-	117%	11	-	246
P3	107%	99%	-	117%	2	-	51
P4	108%	104%	-	113%	2	-	9
P5	107%	104%	-	111%	2	-	47

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Dilution Linearity

Dilution linearity demonstrates that a sample with an analyte concentration above the ULOQ can be diluted to concentration within the assay dynamic range and give a reliable result. Five normal pooled human EDTA plasma was spiked with recombinant NfL and then serially diluted 4x with sample dilution buffer for up to 1024x dilutions. Percent recovery was normalized to the dilution adjusted, neat concentration.

Tested Sample	Starting Conc (pg/mL)	Avg Recovery	Recovery Range		
P1	16648	101%	98%	-	102%
P2	16613	102%	96%	-	112%
P3	17686	99%	95%	-	104%
P4	19331	103%	97%	-	109%
P5	16121	97%	92%	-	103%

Spike Recovery

Spike recovery determines if the concentration–response relationship is similar in the calibration curve/buffer and the samples and is a way to look at matrix effect. A known amount of analyte is spiked into the natural test sample matrix and its response is measured (recovery) in the assay by comparison to an identical amount spiked into the standard diluent.

Spike recovery was determined with 5 EDTA plasma samples which were spiked and compared with spike into sample diluent.

$$\%\ Recovery = \frac{Meas.Conc_{\ Spiked\ Donor\ Sample} - Meas.Conc_{\ Unspiked\ Donor\ Sample}}{Meas.Conc_{\ Spiked\ SD\ buffer}} x100$$

	EDTA Plasma (n = 5)			
Targeted Spike Conc (pg/mL)	Avg Recovery	Recovery Range		
8.2	86%	80%	-	93%
68	95%	88%	-	102%
12750	95%	90%	-	101%

Sample handling recommendations

Samples should be stored at -80°C whenever possible. They should not be left at room temperature for more than 4 hours or at 4°C for more than 48 hours. Additionally, avoid subjecting samples to more than three freeze-thaw cycles.

Ordering Information

NULISAqpcr Assays			
Product Name	Plate Format	Sample Type	Catalog Number
NULISAqpcr NfL (NEFL) Assay	96	plasma/serum	800105

Instrument, Consumables & Buffers		
Product Name	Qty	Catalog Number
Alamar ARGO HT System	1	800101
NULISA Wash Buffer (3L)	3L	801035

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