Quanterix™
The Science of Precision Health

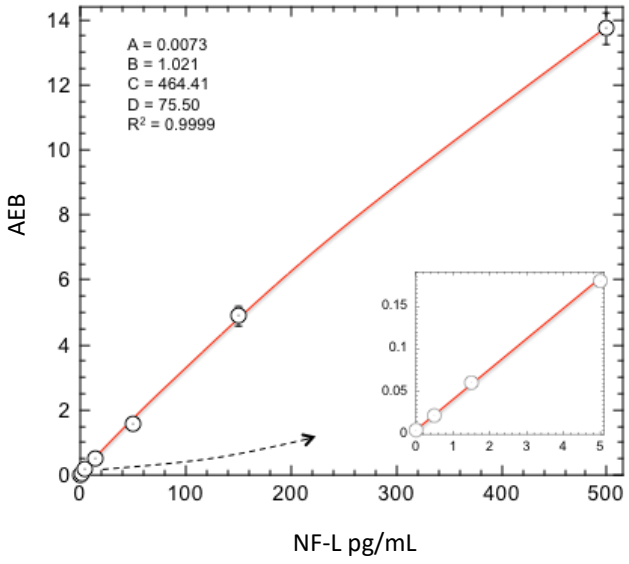
Simoa® NF-light™ Advantage Kit
HD-1/HD-X Data Sheet

Item 103186

Description

Neurofilament light (NF-L) is a 68 kDa cytoskeletal intermediate filament protein that is expressed in neurons. It associates with the 125 kDa Neurofilament medium (NF-M) and the 200 kDa Neurofilament heavy (NF-H) to form neurofilaments. They are major components of the neuronal cytoskeleton, and are believed to function primarily to provide structural support for the axon and to regulate axon diameter. Neurofilaments can be released in significant quantity following axonal damage or neuronal degeneration. NF-L has been shown to associate with traumatic brain injury, multiple sclerosis, frontotemporal dementia and other neurodegenerative diseases. The Simoa NF-light assay is a digital immunoassay for the quantitative determination of NF-L in serum, plasma and CSF. The antibodies (Uman Diagnostics, Umeå Sweden) also cross react with murine, bovine, and macaque NF-L epitopes, and the assay can be used for research with these species.

Calibration Curve: Four-parameter curve fit parameters are depicted.



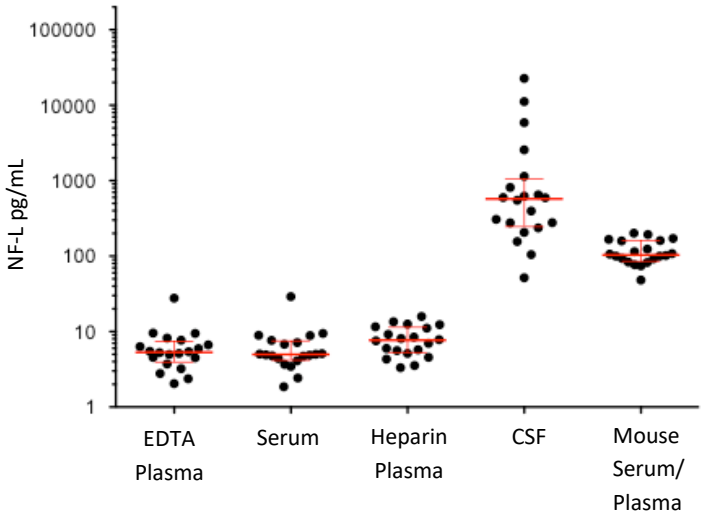
Lower Limit of Quantification (LLOQ): Triplicate measurements of serially diluted calibrator were read back on the calibration curve over 2 reagent lots across 3 instruments (12 runs total).

Limit of Detection (LOD): Calculated as 2.5 standard deviations from the mean of background signal read back on each calibration curve over 2 reagent lots across 3 instruments (12 runs total).

LLOQ	**0.174 pg/mL** pooled CV 15.3% mean recovery 105%
LOD	**0.038 pg/mL** range 0.003–0.079 pg/mL
Dynamic range (serum and plasma)	~1800 pg/mL
Dynamic range (CSF)	~45 ng/mL
Diluted Sample volume*	152 µL per measurement
Tests per kit	96

***See Kit Instruction for details**

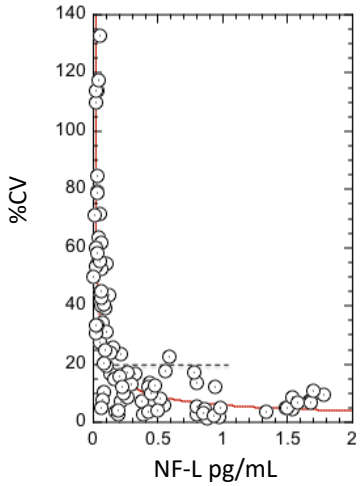
Endogenous Sample Reading: NF-L in EDTA plasma (n=20), matched serum (n=20), heparin plasma (n=20), cerebral spinal fluid (CSF, n=20), and mouse serum and plasma (n=10 each) from non-medicated, non-immunized mice. Error bars depict median and interquartile ranges.



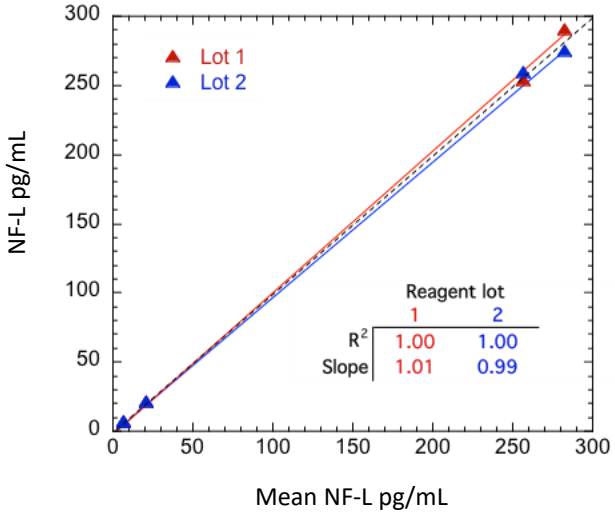
Quanterix Corporation
900 Middlesex Turnpike, Billerica, MA 01821
techsupport@quanterix.com

Sample Type	Mean NF-L pg/mL	Median NF-L pg/mL	% Above LOD
EDTA Plasma	6.56	5.33	100%
Serum	6.62	4.98	100%
Heparin Plasma	8.18	7.69	100%
CSF	2467	572	100%
Mouse Serum/Plasma	118	104	100%

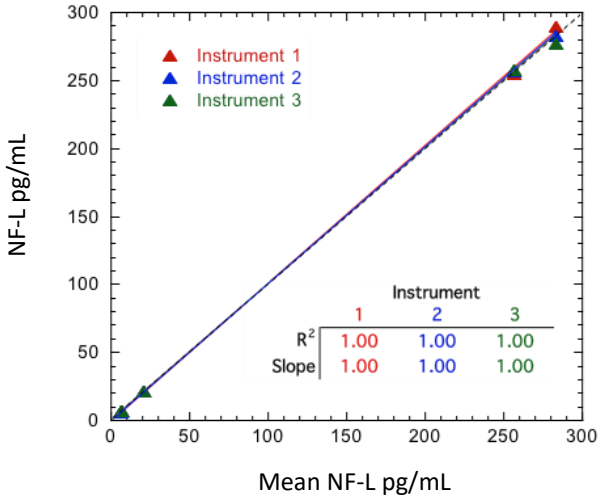
Sample Dose CV Profile: Triplicate measurements of diluted serum samples assayed over multiple runs (90 measurements).



Reproducibility Across Reagent Lots: Five native and spiked serum and plasma NF-L samples tested across 2 runs x 3 instrumentss each lot.



Reproducibility Across Instruments: Five native and spiked serum and plasma NF-L samples tested across 2 runs x 2 reagent lots each instrument.



Reproducibility Precision: Five samples, consisting of serum/plasma panels and NF-L controls, were assayed in replicates of three for two runs on each of three instruments and two reagent lots. Analysis of variance (nested ANOVA) results are summarized in the following table.

Sample	Mean (pg/mL)	Within run CV	Between run CV	Between Lot	Between Instrument
Control 1	6.44	6.5%	4.8%	1.4%	0.0%
Control 2	283	4.7%	5.9%	0.0%	6.4%
Serum Panel 1	6.62	7.5%	3.8%	0.0%	3.8%
Serum Panel 2	21.2	6.7%	4.1%	0.0%	0.0%
Serum Panel 3	257	9.3%	4.3%	0.0%	0.4%

Repeatability Precision: Five samples, consisting of serum/plasma panels and NF-L controls, were assayed in replicates of three at two separate times per day for five days using a single lot of reagents and calibrators. Analysis of variance (nested ANOVA) results are summarized in the following table.

Quanterix Corporation
900 Middlesex Turnpike, Billerica, MA 01821
techsupport@quanterix.com

Sample	Mean (pg/mL)	Within run CV	Between run CV	Between Day
Control 1	6.41	6.6%	0.0%	0.90%
Control 2	255	3.5%	3.1%	7.7%
Serum Panel 1	6.2	6.1%	5.3%	2.7%
Serum Panel 2	21.7	6.8%	1.9%	1.8%
Serum Panel 3	256	7.3%	5.3%	0.0%

Inter Lot CV: Pool of CVs from 5 samples (range: 6.41–256 pg/mL) tested with 2 reagent lots across 2 runs x 3 instruments.

Inter Instrument CV: Pool of CVs from 5 samples (range: 6.41–256 pg/mL) tested with 3 instruments across 2 runs x 2 reagent lots.

Inter Lot CV	**1.74%**
Inter Instrument CV	**2.07%**

Spike and Recovery: Pool of CSF samples spiked into 5 serum and 5 plasma samples.

Admixture Linearity: High NF-L plasma sample fractionally admixed with low NF-L serum sample, mean of 10 levels.

Dilution Linearity (Serum/Plasma): Diluted 2x serially from MRD (4x) to 128 MRD (512x) with Sample Diluent.

Dilution Linearity (CSF): 2 CSF samples diluted 2x serially from MRD (100x) to 128 MRD (12800x) with Sample Diluent.

Spike and Recovery (Serum/Plasma)	**Mean = 68.3%** Range: 59.7–80.4%
Admixture Linearity	**Mean = 97.0%** Range: 88.2–105%
Dilution Linearity (Serum/Plasma, 512x)	**Mean = 101%** Range: 82.4–178%
Dilution Linearity (CSF, 12800x)	**Mean = 100%** Range: 94.1–106%

Specificity (Healthy Normal CSF and Plasma): 99.0%. 5 of each type depleted with capture antibody, grand mean.

Specificity (ALS CSF and Plasma): 99.6%. 5 of each type depleted with capture antibody, grand mean.

The Simoa NF-light Advantage assay kit is formulated for use on the Quanterix SR-X™, Simoa HD-1®, or Simoa HD-X Analyzer® platform. Data in this document was obtained from runs on the HD-1 platform unless otherwise noted. Some differences in performance claims between SR-X and HD-1/HD-X may be observed when comparing datasheets for these platforms. This may be due to experiments run at different time-points with different reagent lots and different samples, or it may be due to minor differences in antibody and analyte behavior in the different assay formats.

Quanterix Corporation
900 Middlesex Turnpike, Billerica, MA 01821
techsupport@quanterix.com