Simoa® NF-light™ V2 Advantage Kit
HD-1/HD-X Data Sheet

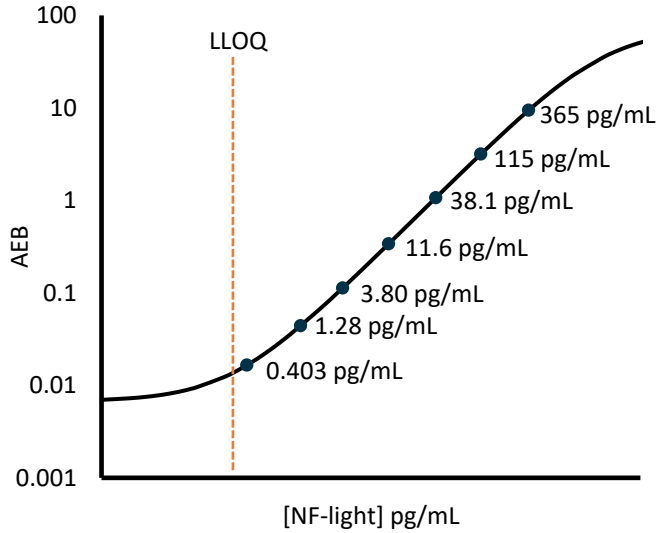
Item 104073

Description

This datasheet summarizes data from analytical validation performed at Quanterix to characterize performance of the NF-light V2 Advantage kit on the HD-1/HD-X platform.

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: Representative calibrator concentrations and Lower Limit of Quantification (LLOQ) depicted. Concentrations of Reference Calibrators provided with individual kit lots will vary, as they are value assigned to maintain consistent calibration and sample readings across lots. For NF-light V2 Reference Calibrator sets, the minimum allowable concentration for Cal H is 360 pg/mL and the maximum allowable concentration for Cal B is 0.65 pg/mL.



Minimum Required Dilution (MRD)

Diluted Sample Volume	100 µL per measurement
Human Serum and EDTA Plasma Dilution	1:4
Human CSF Dilution	1:100
Tests per kit	96

See Kit Instruction for details.

Lower Limit of Quantification (LLOQ): Triplicate measurements of serially diluted calibrator were read back on the calibration curve over 12 runs across 2 reagent lots and 2 instruments (3 runs per lot, per instrument). The analytical LLOQ was set at the lowest concentration that read back within 80 – 120% of the expected value with a CV < 20%. The functional LLOQ values represent the analytical LLOQ multiplied by the dilution factor used for the samples. The functional LLOQ for plasma/serum and CSF are 4x and 100x the analytical LLOQ, respectively.

Kit Release Lower Limit of Quantification (LLOQ): The Kit Release LLOQ is a criterion applied during QC testing of each kit lot; it specifies the highest acceptable value for LLOQ. For QC testing, LLOQ is determined from HD-X or HD-1 data using the CV Profiling method described in the Technical Note, ***Lower Limit of Quantification*** (TECH-0034), available on the Quanterix Customer Portal. This is an analytical LLOQ and must be

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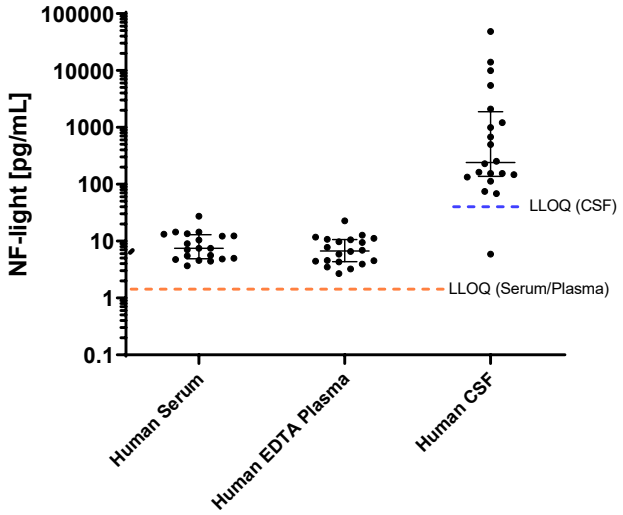
multiplied by the sample dilution factor to convert to functional LLOQ.

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

Assay Range: The upper end of the dynamic range, or functional Upper Limit of Quantification (ULOQ) is equal to the minimum top calibrator concentration multiplied by MRD. Note that the concentration of the highest calibrator will vary between kit lots, as Reference Calibrators are value assigned to maintain consistency of results across lots.

Analytical LLOQ	**0.345 pg/mL** pooled CV 18.9% mean recovery 110.9%
Functional LLOQ (serum and plasma)	**1.38 pg/mL**
Functional ULOQ (serum and plasma)	**1440 pg/mL**
Functional LLOQ (CSF)	**34.5 pg/mL**
Functional ULOQ (CSF)	**36000 pg/mL**
LOD	**0.085 pg/mL** range 0.026-0.162 pg/mL
Kit Release LLOQ	**0.539 pg/mL**

Endogenous Sample Reading: Healthy donor matched human serum and EDTA plasma (n=20), and unmatched normal human CSF (n=20) were measured. Bars depict median with interquartile range. Orange line represents functional LLOQ.



Sample Type	Mean NF-light pg/mL	Median NF-light pg/mL	% Above LOD	% Above LLOQ
Human Serum	9.38	7.47	100%	100%
Human EDTA Plasma	7.88	6.74	100%	100%
Human CSF	4475	241	100%	95%

*** Values below LLOQ are not included in the mean**

Precision: Measurements of 1 human serum-based panel, 3 human EDTA plasma-based panels and 2 calibrator-based controls. Triplicate measurements were made for 12 runs each for 2 reagent lot across 2 instruments (12 runs total, 36 measurements). All samples were diluted at the appropriate MRD for the sample matrix.

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Sample	Mean (pg/mL)	Within run CV	Between run CV	Between inst CV	Between Lot CV
Control 1	3.67	8.5%	9.7%	0.5%	6.0%
Control 2	153	3.0%	9.8%	1.7%	10.2%
Panel 1	11.1	6.4%	7.2%	2.6%	3.1%
Panel 2	8.24	5.9%	6.1%	2.2%	0.1%
Panel 3	89.7	2.5%	9.9%	1.9%	11.1%
Panel 4	271	3.1%	10.8%	5.9%	10.4%

Spike and Recovery: Two samples each of human serum and human EDTA plasma, were spiked with recombinant antigen at high and low concentrations within the range of the assay and analyzed on HD-X. The same serum and plasma donor samples were used to test spike and recovery across two different lots of NF-Light V2 assay kits. CSF samples were tested similarly, but with 2 different donor samples spiked for analysis with each of the 2 NF-Light V2 assay kit lots. Percent recovery is defined as the difference between the measured concentration of NF-light in the spiked sample and the measured concentration in unspiked sample relative to the concentration of NF-light in spiked calibrator diluent. Results indicate that Matrix effects are observed with this assay, especially when spiking recombinant antigen into plasma and serum samples.

Dilution Linearity: Two samples each of human EDTA plasma and human serum were spiked with human CSF samples and serially diluted with sample diluent through 7 levels of 2X dilutions. Each dilution series was run on the HD-X with two different lots of NF-Light V2 assay kits with the MRD (4x) dilution applied. Total dilution of each plasma or serum sample ranged from 4x to 512x. Three human CSF samples were serially diluted with sample diluent through up to 7 levels of 2x dilutions. CSF donor 1 was tested with two NF-Light V2 assay kit lots, whereas CSF donors 2 and 3 were each tested with a single NF-Light V2 assay kit lot. Each CSF dilution series was run on the HD-X with the MRD (100x) dilution applied. Total dilution of each sample ranged from 100x to up to 12800x.

Spike and Recovery (Human Serum)	**Mean 39%** range 24-55%
Spike and Recovery (Human EDTA Plasma)	**Mean 53%** range 40–65%
Spike and Recovery (Human CSF)	**Mean 77%** range 66–87%
Dilution Linearity (Human Serum, 4x - 512x)	**Mean 105%** range 76–125%
Dilution Linearity (Human EDTA Plasma, 4x - 512x)	**Mean 100%** range 81–132%
Dilution Linearity (Human CSF donor 1, 100x - 3200x)	**Mean 106%** range 96–115%
Dilution Linearity (Human CSF donor 2, 100x – 12800x)	**Mean 96%** range 92–100%
Dilution Linearity (Human CSF donor 3, 100x – 12800x)	**Mean 103%** range 95–107%

The Simoa NF-light V2 advantage assay kit is formulated for use on the SR-X, HD-1, or HD-X platform. Some differences in performance claims between the HD and SR-X platforms may be observed when comparing data sheets for these platforms. This may be due to experiments run at different time-points with different reagent lots and different samples or may be due to minor differences in antibody and analyte behavior in the different assay formats.

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