Quanterix™
The Science of Precision Health

Simoa® Neurology 2-Plex B Kit
HD-1/HD-X Data Sheet

Item 103520

Description

The Simoa Human Neurology 2-Plex B assay (N2PB) measures two important neurology biomarkers in both cerebrospinal fluid (CSF) and blood. The two targets are neurofilament light (NF-light) and glial fibrillary acidic protein (GFAP). Both biomarkers have been studied as indicators of traumatic brain injury (TBI) severity.

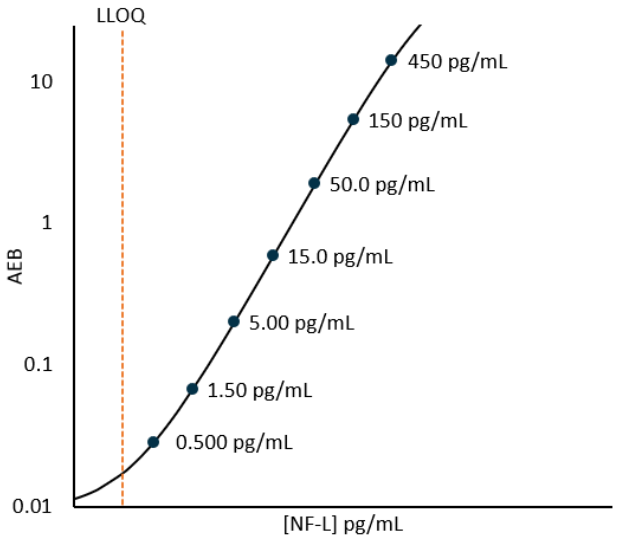
Description – NF-light Test

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.

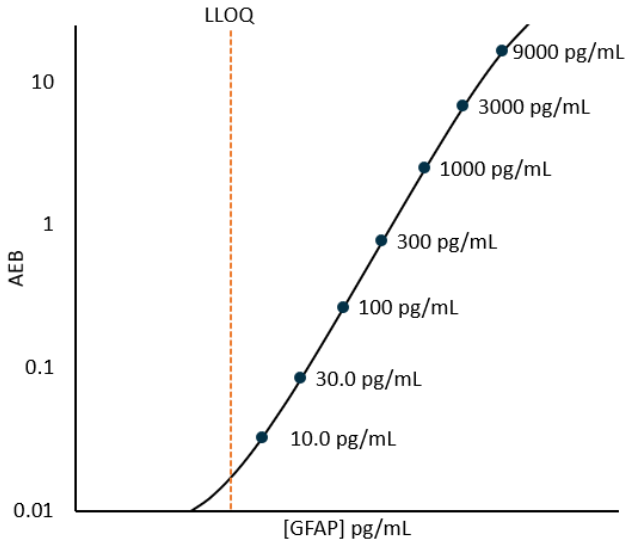
Description – GFAP Test

Glial Fibrillary Acidic Protein (GFAP) is a class-III intermediate filament majorly expressed in astrocytic glial cells in the central nervous system. Astrocytes play a variety of key roles in supporting, guiding, nurturing, and signaling neuronal architecture and activity. Monomeric GFAP is about 55kD. It is capable of forming both homodimers and heterodimers; GFAP can polymerize with other type III proteins or with neurofilament protein (such as NF-L). GFAP is involved in many important CNS processes, including cell communication and the functioning of the blood brain barrier. GFAP, as a potential biomarker has been shown to associate with multiple diseases such as traumatic brain injury, stroke, brain tumors, etc. Decreases in GFAP expression have been reported in Down's syndrome, schizophrenia, bipolar disorder, and depression.

NF-L Curve: Calibrator concentrations and Lower Limit of Quantification depicted.



GFAP Curve: Calibrator concentrations and Lower Limit of Quantification depicted.



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Minimum Required Dilution (MRD)

Diluted Sample Volume	100 µL per measurement
Serum and Plasma Dilution	1:4
CSF Dilution	1:40

See Kit Instruction for details.

Lower Limit of Quantification (LLOQ): Triplicate measurements of serially diluted calibrator were read back on the calibration curve over 3 runs each for 2 reagent lots across 2 instruments (6 runs total). The functional LLOQ (fLLOQ) values below are for serum and plasma. The fLLOQ for CSF is 10X the fLLOQ for serum and plasma.

	Analytical LLOQ	Functional LLOQ (x MRD)
NF-light	**0.200 pg/mL** pooled CV 18% mean recovery 114%	**0.800 pg/mL**
GFAP	**4.15 pg/mL** pooled CV 16% mean recovery 107%	**16.6 pg/mL**

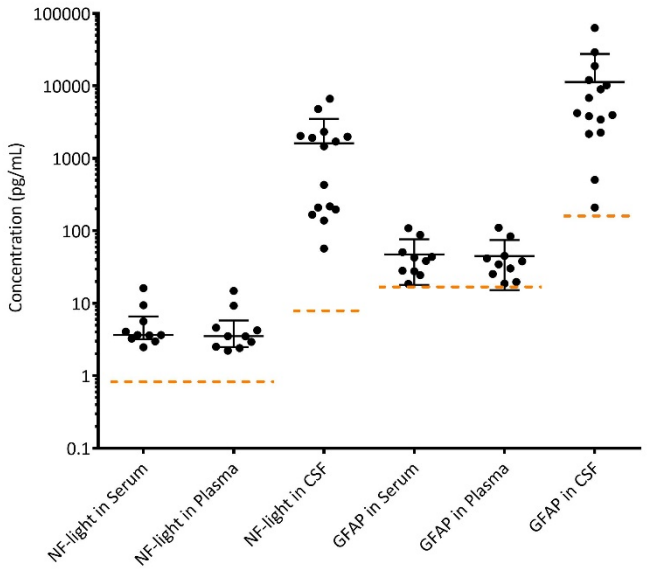
Limit of Detection (LOD): Calculated as 2.5 standard deviations from the mean of background signal read back on each calibration curve over 3 runs each for 2 reagent lots across 2 instruments (6 runs total).

	LOD
NF-light	**0.065 pg/mL** range 0.040-0.098 pg/mL
GFAP	**0.475 pg/mL** range 0.135-0.826 pg/mL

Assay Range: The upper end of the dynamic range is equal to the top calibrator concentration multiplied by MRD. The ranges below are for serum and plasma. The Upper Limit of Quantification (ULOQ) for CSF is 10X the ULOQ for serum and plasma.

	Assay Range
NF-light	0 - ~2000 pg/mL
GFAP	0 - ~40000 pg/mL

Endogenous Sample Readings: Healthy donor matched EDTA plasma and serum (n=10) and CSF (n=15) were measured. Bars depict median with interquartile range. Orange line represents functional LLOQ.



	Sample Type	Mean pg/mL	Median pg/mL	% Above LOD	% Above LLOQ
NF-light	Serum	6.20	4.22	100%	100%
	Plasma	5.38	3.63	100%	100%
	CSF	951	1343	100%	100%
GFAP	Serum	47.1	36.8	100%	100%
	Plasma	42.6	36.9	100%	95%
	CSF	8626	3607	100%	100%

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Precision: Measurements of 4 serum- or plasma-based panels, 1 CSF panel, and 2 calibrator-based controls. Triplicate measurements were made for 3 runs each for 2 reagent lots across 2 instruments (6 runs total).

NF-light	Mean pg/mL	Within run CV	Between run CV	Between lot CV
Control 1	4.44	8.1%	4.2%	1.1%
Control 2	582	4.2%	6.3%	2.1%
Panel 1	3.35	5.8%	6.1%	13.7%
Panel 2	7.25	5.2%	5.3%	4.6%
Panel 3	97.8	2.5%	5.9%	9.1%
Panel 4	459	4.2%	5.0%	4.5%
CSF Panel	8549	3.8%	12.5%	4.9%

GFAP	Mean pg/mL	Within run CV	Between run CV	Between lot CV
Control 1	93.4	5.0%	0.7%	0.9%
Control 2	12434	3.6%	6.1%	2.2%
Panel 1	51.8	4.9%	12.0%	4.6%
Panel 2	54.0	7.2%	3.0%	8.2%
Panel 3	1332	2.6%	2.7%	4.1%
Panel 4	4705	5.4%	5.6%	8.2%
CSF Panel	7403	4.0%	12.9%	0.5%

Note: Data in the following sections were obtained using the N4PB assay.

Spike and Recovery: 9 serum, 9 EDTA plasma, and 4 CSF samples were spiked at high and low concentrations within the range of the assay.

	Sample Type	Recovery
NF-light	Serum/Plasma	**71%** range 58-83%
	CSF	**117%** range 108-138%
GFAP	Serum/Plasma	**52%** range 38-66%
	CSF	**120%** range 103-134%

Dilution Linearity: 5 spiked serum and 5 spiked plasma samples were diluted 2X serially from MRD (4X) to 64X with Sample Diluent. 2 spiked CSF samples were diluted 2X serially from MRD (40X) to 640X with Sample Diluent.

	Sample Type	Linearity
NF-light	Serum/Plasma	**117%** range 47-139%
	CSF	**84%** range 81-87%
GFAP	Serum/Plasma	**141%** range 63-187%
	CSF	**94%** range 93-94%

The Simoa N2PB assay kit is formulated for use on the SR-X, HD-1, or HD-X platform. Some differences in performance claims between the HD-1/X 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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