Simoa® Neurology 4-Plex E Advantage Kit
HD-X® Data Sheet

Item 103670

Human Neurology 4-Plex E assay (N4PE)

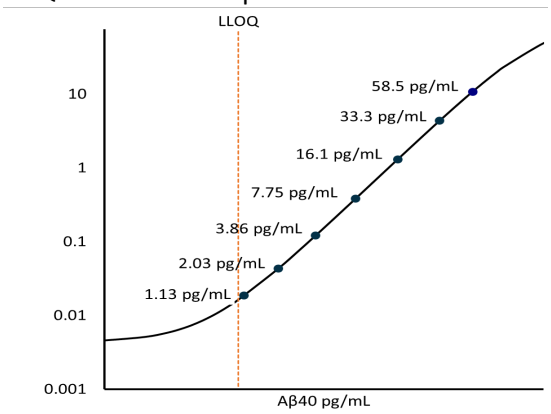
The Simoa Human Neurology 4-Plex E (N4PE) assay simultaneously measures four biomarkers in EDTA plasma and CSF. The targets are Abeta 40 (Aβ40), Abeta 42 (Aβ42), Glial Fibrillary Acidic Protein (GFAP), and Neurofilament light (NF-L). Each biomarker has been demonstrated to have significance for the investigation of neurodegeneration and brain injury, and to have utility for monitoring effectiveness of drug discovery and development for neurological disease.

With this Neuro 4-Plex E kit we introduce novel Abeta 40 and Abeta 42 assays developed for highly specific and sensitive measurement of the concentrations of full length Abeta1-42 and Abeta1-40[1]. This improved specificity for full-length Abeta1-42 and Abeta1-40 is expected to be increasingly important for monitoring the effect of treatments that target specific Abeta isoforms and for target engagement in future clinical trials.

Description – Aβ40 Test

Aβ40 is a 40 amino acid proteolytic product from the amyloid precursor protein (APP) that has gained attention as a biomarker correlating with Alzheimer disease (AD) onset, mild cognitive impairment, vascular dementia, and other cognitive disorders. Beta-secretase cleavage of APP initially results in the production of an APP fragment that is further cleaved by gamma-secretase at residues 40-42 to generate two main forms of amyloid beta, Aβ40 and Aβ42. Amyloid beta (Aβ) peptides (including a shorter Aβ38 isoform) are produced by different cell types in the body, but the expression is particularly high in the brain. Accumulation of Aβ in the form of extracellular plaques is a neuropathological hallmark of AD and believed to play a central role in the neurodegenerative process. Aβ40 is the major amyloid component in these plaques and is thought to be an initiating factor of AD plaques. In healthy and disease states Aβ40 is the most abundant form of the amyloid peptides in both cerebrospinal fluid (CSF) and plasma (10–20X higher than Aβ42). Recent studies suggest that a decrease in the ratio of Aβ42/Aβ40 may indicate AD progression. Determinations in serum samples are not reported due to high variability of Aβ40 in some healthy donor sample sets.

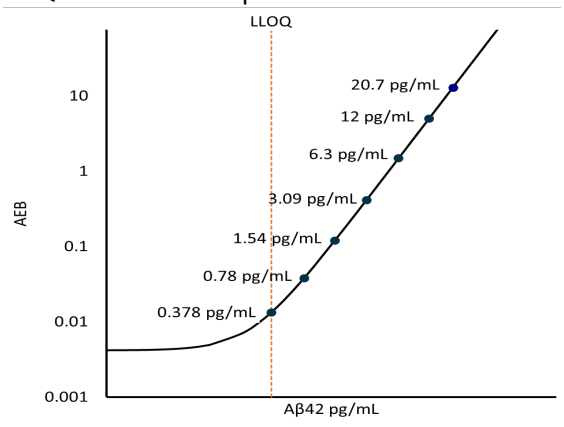
Aβ40 Curve: Calibrator concentrations and Lower Limit of Quantification depicted.



Description – Aβ42 Test

Aβ42 is a 42 amino acid proteolytic product from the amyloid precursor protein that has gained considerable attention as a biomarker correlating with Alzheimer disease (AD) onset, mild cognitive impairment, vascular dementia, and other cognitive disorders. Amyloid beta (Aβ) peptides (including the shorter Aβ38 and Aβ40 isoforms) are produced by many cell types in the body but the expression is particularly high in the brain. Accumulation of Aβ in the form of extracellular plaques is a neuropathological hallmark of AD and thought to play a central role in the neurodegenerative process. Substantial clinical validation has now been developed around disease relevance of cerebrospinal fluid (CSF) levels of Aβ42, and there follows a significant interest in measuring blood levels of this marker. Concentrations of Aβ42 in blood are over 100-fold lower than in cerebrospinal fluid, (typically single pg/mL range), requiring very high analytical sensitivity for its reliable measurement. Determinations in serum samples are not reported due to high variability of Aβ42 in some healthy donor sample sets.

Aβ42 Curve: Calibrator concentrations and Lower Limit of Quantification depicted.



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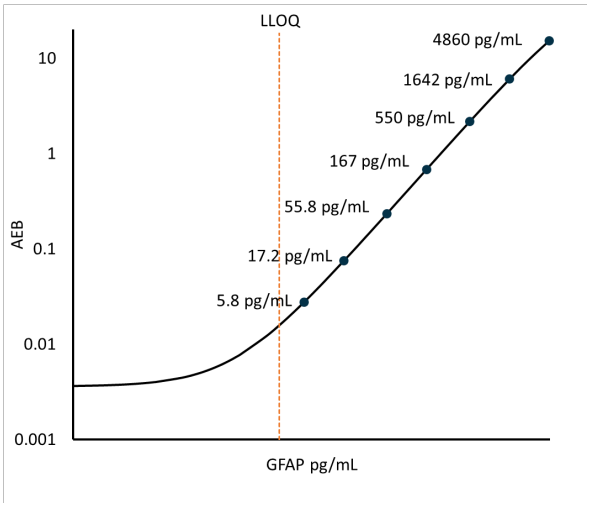
Description – GFAP Test

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 can form 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. As a potential biomarker, GFAP 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.

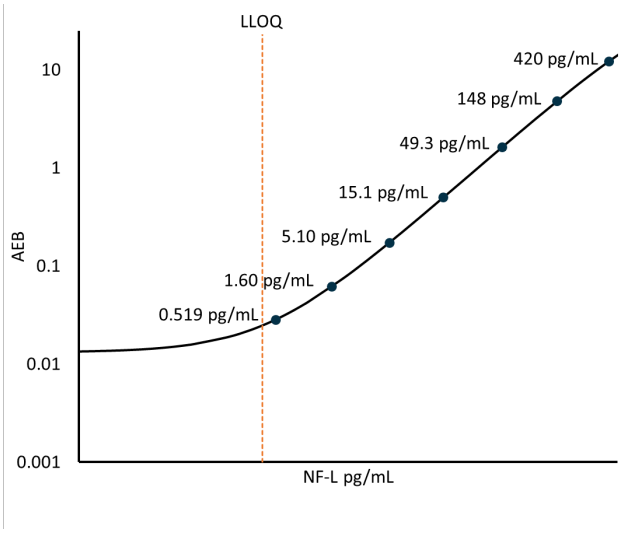
GFAP Curve: Calibrator concentrations and Lower Limit of Quantification depicted.



Description – NF-L 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.

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



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Minimum Required Dilution (MRD)

Diluted Sample Volume	100 µL per measurement
Plasma Dilution	1:4
CSF Dilution	1:400
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 6 runs each for 2 reagent lots across 2 instruments (12 runs total). The functional LLOQ (fLLOQ) values below are for plasma. The fLLOQ for CSF is 100x the fLLOQ for plasma.

	Analytical LLOQ	Functional LLOQ (x MRD)
Aβ40	**1.02 pg/mL** pooled CV 8.0% mean recovery 110%	**4.08 pg/mL**
Aβ42	**0.378 pg/mL** pooled CV 6.6% mean recovery 102%	**1.51 pg/mL**
GFAP	**2.89 pg/mL** pooled CV 14.2% mean recovery 90.7%	**11.6 pg/mL**
NF-L	**0.400 pg/mL** pooled CV 14.2% mean recovery 100%	**1.6 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 6 runs each for 2 reagent lots across 2 instruments (12 runs total).

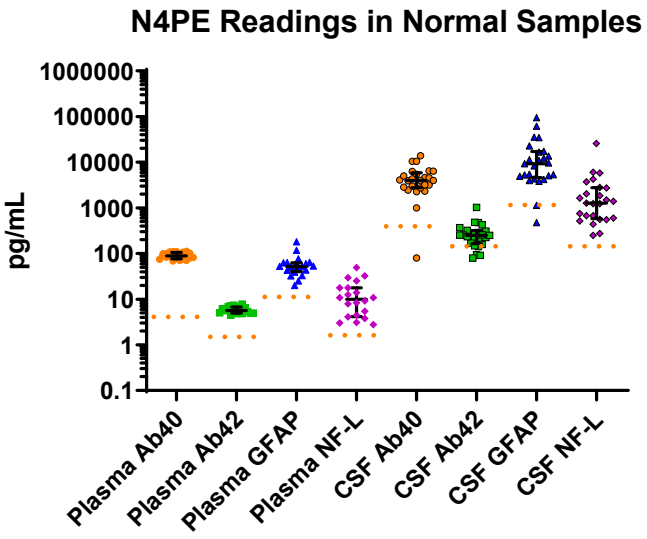
	LOD
Aβ40	**0.384 pg/mL** range 0.189 - 0.531 pg/mL
Aβ42	**0.136 pg/mL** range 0.0601 - 0.204 pg/mL
GFAP	**0.441 pg/mL*** range 0.240 - 0.757 pg/mL
NF-L	**0.090 pg/mL** range 0.016-0.152 pg/mL

*Determined from 11 runs

Assay Range: The upper end of the dynamic range is equal to the top calibrator concentration multiplied by MRD. The ranges below are for plasma. The Upper Limit of Quantification (ULOQ) for CSF is 100x the ULOQ for plasma.

	Assay Range
Aβ40	0 - 280 pg/mL
Aβ42	0 - 100 pg/mL
GFAP	0 – 20,000 pg/mL
NF-L	0 – 2,000 pg/mL

Endogenous Sample Reading: Healthy donor EDTA plasma (n=20), and CSF (n=25) were measured. Bars depict median with interquartile range. Orange line represents functional LLOQ.



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	Sample Type	Mean Conc pg/mL	Median Conc pg/mL	% Above LOD	% Above LLOQ
Aβ40	Plasma	90.9	89.4	100%	100%
	CSF	4862	3959	96%	96%
Aβ42	Plasma	5.87	5.69	100%	100%
	CSF	321	250	100%	80%
GFAP	Plasma	58.8	52.2	100%	100%
	CSF	17426	9295	100%	92%
NF-L	Plasma	13.6	10.0	100%	100%
	CSF	2743	1268	100%	100%

Precision: Measurements of 3 plasma and 1 CSF based panels, and 2 calibrator-based controls. Triplicate measurements were made for 6 runs each for 2 reagent lot across 2 instruments (12 runs total, 36 measurements).

Aβ40	Mean (pg/mL)	Within run CV	Between run CV	Between inst CV	Between Lot CV
Control 1	14.6	2.0%	2.3%	0.1%	0.25%
Control 2	107	3.4%	2.4%	0.49%	1.61%
Panel 1	14.7	3.1%	2.6%	1.0%	1.2%
Panel 2	52.0	1.6%	3.1%	0.2%	1.1%
Panel 3	127	2.6%	3.8%	0.8%	2.4%
Panel 4	6848	2.0%	4.6%	0.7%	3.5%

Aβ42	Mean (pg/mL)	Within run CV	Between run CV	Between inst CV	Between Lot CV
Control 1	5.43	2.3%	2.9%	1.5%	0.72%
Control 2	29	3.1%	3.3%	0.01%	2.87%
Panel 1	1.37	8.7%	11.0%	2.9%	8.2%
Panel 2	20.3	1.4%	4.2%	0.1%	1.3%
Panel 3	43	2.5%	3.3%	0.3%	0.7%
Panel 4	468	2.7%	8.2%	1.3%	0.9%

GFAP	Mean (pg/mL)	Within run CV	Between run CV	Between inst CV	Between Lot CV
Control 1	215	3.0%	5.0%	2.2%	4.91%
Control 2	4358	5.8%	4.2%	2.48%	2.03%
Panel 1	26.7	9.3%	14.4%	4.9%	4.1%
Panel 2	498	2.9%	11.2%	1.4%	8.2%
Panel 3	2151	4.5%	9.0%	1.2%	5.8%
Panel 4*	5783	5.0%	7.5%	1.2%	0.7%

***Avoid freeze/thaw cycles with CSF samples**

NF-L	Mean (pg/mL)	Within run CV	Between run CV	Between inst CV	Between Lot CV
Control 1	20.1	4.5%	5.2%	1.8%	5.62%
Control 2	410	5.3%	6.4%	3.62%	1.48%
Panel 1	6.29	8.1%	7.2%	1.3%	5.9%
Panel 2	70.0	2.9%	7.2%	1.5%	7.8%
Panel 3	360	4.4%	7.5%	2.3%	5.4%
Panel 4	299	10.8%	10.3%	4.4%	0.2%

Spike and Recovery: 2 CSF and 2 EDTA plasma samples were spiked at high and low concentrations within the range of the assay and analyzed on HD-X.

	CSF Recovery	Plasma Recovery
Aβ40	**94.9%** range 86.7-106%	**67.8%** range 61.8-72.9%
Aβ42	**90.8%** range 85.4-95.0%	**71.7%** range 65.4-75.7%
GFAP	**91.4%** range 84.4-96.7%	**58.1%** range 49.5-64.8%
NF-L	**88.7%** range 80.6-93.4%	**111%** range 94.9-120%

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Dilution Linearity: 2 endogenous and 2 spiked EDTA plasma were diluted 2x serially from MRD (4x) and 2 endogenous and 2 spiked CSF samples were diluted 2x serially from MRD (400x) to indicated dilution factor with Sample Diluent.

Linearity				
	Matrix	**Source**	**Dilution Factor**	**Results**
Aβ40	Plasma	Endogenous	8	112%
	Plasma	Spiked	32	101%
	CSF	Endogenous	800	92%
	CSF	Spiked	12,800	88%
Aβ42	Plasma	Endogenous	8	93%
	Plasma	Spiked	32	101%
	CSF	Endogenous	800	99%
	CSF	Spiked	12,800	107%
GFAP	Plasma	Endogenous	8	117%
	Plasma	Spiked	32	118%
	CSF	Endogenous	800	102%
	CSF	Spiked	12,800	93%
NF-L	Plasma	Endogenous	8	111%
	Plasma	Spiked	32	97%
	CSF	Endogenous	800	91%
	CSF	Spiked	12,800	92%

[1]Thijssen et al. Highly Speci□c and Ultrasensitive Plasma Test Detects Abeta(1-42) and Abeta(1-40) in Alzheimer's Disease. Alzheimer's Research & Therapy. 2020: In Press.

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