Quanterix™
The Science of Precision Health

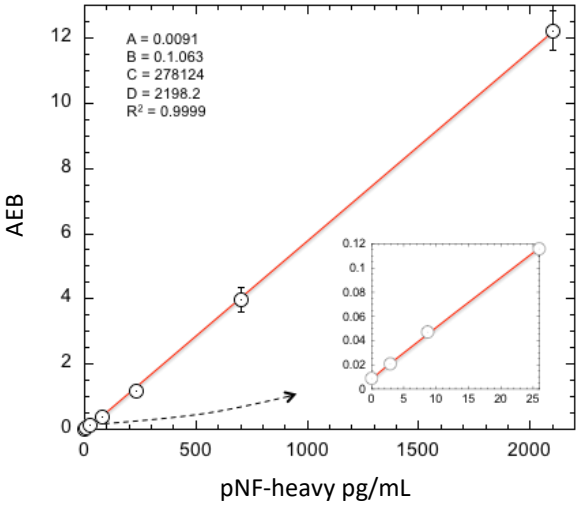
Simoa® pNF-heavy Discovery Kit
HD-1/HD-X Data Sheet

Item 102669

Description

Neurofilaments (NF) are the main cytoskeletal constituents in neuronal cells. Their primary function is to maintain the diameter of axons and dendrites and transmit electrical impulses along axons. CNS Neurofilaments are heteropolymers composed of 4 subunits: NF-light (~68kDa), NF-medium (~150kDa), NF-heavy (~200kDa), and alpha-internexin. The NF-heavy and NF-medium C-terminal domains have multiple tandem 6-8 amino acid sequence repeats containing conserved KSXXP motifs. The serine residue is the major axonal phosphorylation site. Dendritic and perikaryal NF-heavy is not phosphorylated on these sites, therefore a release of phosphorylated NF-heavy can be used as a biomarker of axonal damage and degeneration. pNF-heavy is a cytoskeletal protein present in abundant amounts and consequently detectable once released into the blood, is highly immunogenic, and protease resistant. These characteristics make pNF-heavy a desirable CNS biomarker. Recent studies have shown that when compared with healthy controls, mild Traumatic Brain Injury (mTBI) patients have elevated serum levels of pNF-heavy on Days 1 and 3 after TBI. Elevated serum levels of pNF-heavy have been seen in human subjects with acute ischaemic stroke, acute brain injury after cardiac arrest, and Amyotrophic Lateral Sclerosis. Elevated CSF levels have also been demonstrated in animal models of spinal cord injury and brain injury.

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



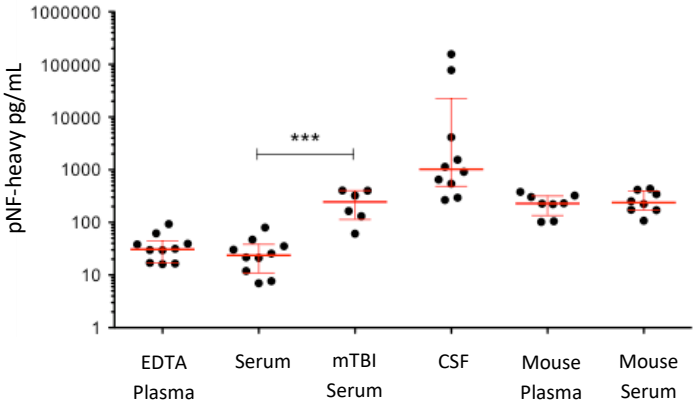
Lower Limit of Quantification (LLOQ): Triplicate measurements of serially diluted calibrator were read back on the calibration curve over 1 reagent lot on 1 instrument (5 runs total).

Limit of Detection (LOD): Calculated as 2.5 standard deviations from the mean of background signal read back on each calibration curve over 1 reagent lot on 1 instrument (5 runs total).

LLOQ	**2.88 pg/mL** pooled CV 10% mean recovery 119%
LOD	**0.663 pg/mL** range 0.12–1.54 pg/mL
Dynamic range (serum and plasma)	0–8400 pg/mL
Diluted Sample volume*	100 µL per measurement
Tests per kit	192

***See Kit Instruction for details**

Endogenous Sample Reading: Healthy donor EDTA plasma (n=10), matched serum (n=10), mild TBI (n=6), and cerebral spinal fluid (n=10) were measured. Mouse plasma (n=8) and mouse serum (n=8) from non-medicated, non-immunized mice were measured. Error bars depict median with interquartile range.



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Sample Type	Mean pNF-heavy pg/mL	Median pNF-heavy pg/mL	% Above LOD
Serum	28.88	23.73	100%
EDTA Plasma	37.39	30.82	100%
mTBI Serum	247.9	244.7	100%
CSF	24406	1025.4	100%
Mouse Plasma	237.1	229.1	100%
Mouse Serum	265.4	237.9	100%

Precision: Representative precision was estimated with repeated assay of serum panels using one instrument and one reagent lot. Within-run and between-run CVs are depicted in the following table. Within-run CVs reflect average CVs across 5 experiments of 3 replicates each.

Sample	Mean (pg/mL)	Within run CV	Between run CV
Plasma Panel 1	2744	4.0%	4.2%
Plasma Panel 2	214	3.4%	10.6%
Serum Panel 3	197	2.7%	9.9%
Plasma Panel 4	37.8	10.1%	15.6%

Spike and Recovery: pNF-heavy spiked into 2 serum and 5 plasma samples at 2 levels.

Dilution Linearity (Serum): Serum diluted 2x serially from MRD (4x) to 64x with Sample Diluent.

Dilution Linearity (CSF): CSF sample diluted 2x serially from MRD (25x) to 800x with Sample Diluent.

Dilution Linearity (Mouse Serum): Serum diluted 2x serially from MRD (10x) to 80x with Sample Diluent.

Spike and Recovery (Serum/Plasma)	**Mean = 92.0%** Range: 11.4–146%
Dilution Linearity (Serum, 64x)	**Mean = 96.2%** Range: 90.5–102%
Dilution Linearity (CSF, 800x)	**Mean = 84.2%** Range: 79.4–90.8%
Dilution Linearity (Mouse Serum, 80x)	**Mean = 109%** Range: 97.3–120%