SMC® Human Neurofilament Light (NF-L) Immunoassay Kit Verification Summary

Analyte: Neurofilament Light (NF-L)

Species: Human

Kit Catalog # 03-0202-00

For Research Use Only. Not for use in diagnostic procedures.

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Contents

1. Summary Results

Limit of Detection (LOD)	0.22 pg/mL
Lower Limit of Quantification (LLoQ)	0.87 pg/mL
Upper Standard Curve Limit	1,500 pg/mL
Total Samples Tested	5 serum, 5 plasma samples, and 5 CSF samples
Endogenous Sample Range	Serum: 1.85 – 21.89 pg/mL, Avg 8.13 pg/mL Plasma: 1.37 – 3.82 pg/mL, Avg 2.72 pg/mL CSF: 85.38 – 180.27 pg/mL, Avg 97.48 pg/mL
Spike Recovery Range	Serum: 77 – 105% Plasma: 78 - 104% CSF: 99 - 165%
Spike Recovery Mean	Serum: 93% Plasma: 94% CSF: 123%
Linearity Range Tested (Serum, Plasma)	1:4, 1:8, 1:16
Linearity Range	Serum: 98 – 119% Plasma: 90 – 115%
Linearity Mean	Serum: 110% Plasma: 106%
Parallelism Range Tested (CSF)	1:8, 1:16, 1:32
Parallelism Range	CSF: 85 – 114%
Parallelism Mean	CSF: 101%
Intra-assay CV% (average of five samples on 3 plates, by 3 operators)	Mean CV 10.6%
Inter-assay CV% (five samples on 3 plates, by 3 operators)	Mean CV 9.6%
Standard Curve	1,500 – 0.43 pg/mL
Sample Volume (Serum, Plasma)	50 µL (diluted 2-fold in standard diluent)
Sample Volume (CSF)	25 µL (diluted 4-fold in standard diluent)
Species	Human
Matrix	K2 EDTA Plasma, Serum, CSF

2. Kit Protocol

The SMC® Human NF-L Immunoassay Kit 03-0202-00 was run according to the package insert.

Subsequent revisions of the kit and/or the package insert may have occurred or will occur. These changes are immaterial to the presented experimental data.

Data was analyzed using SMCxPRO® Software

3. Representative Standard Curve

The range is from 1,500 pg/mL to 0.43 pg/mL, with a lower limit of quantification (LLoQ) of 0.87 pg/mL as indicated by the highlighted row.

LLoQ = back interpolation of standard that provides CV≤ 20% and recovery ± 20% of the expected.
LoD = 2 x (SD zero calibrator) / (slope of calibration curve).

Expected [NF-L] pg/mL	n	Response Mean	SD	CV	Bkgd	LLOQ pg/mL	Slope RE/ pg/mL	LoD pg/mL	Mean [NF-L] pg/mL	SD	CV	Recovery
1500.00	3	4514	86.3	2%	9.79	0.87	8.24	0.02	1499.71	44.8	3%	100%
500.00	3	2551	75.7	3%					470.78	22.0	5%	94%
166.67	3	1206	65.3	5%					172.44	11.0	6%	103%
55.56	3	409	33.1	8%					52.95	4.5	8%	95%
27.78	3	242	6.6	3%					30.88	0.9	3%	111%
13.89	3	104	6.8	7%					12.88	0.9	7%	93%
6.94	3	65	4.3	7%					7.73	0.6	7%	111%
3.47	3	35	4.9	14%					3.71	0.7	18%	107%
1.74	3	21	0.8	4%					1.74	0.1	7%	100%
0.87	2	14	0.1	1%					0.73	0.0	2%	84%
0.43	2	11	0.8	7%					0.32	0.1	37%	74%
0.00	2	10	0.1	1%					-	-	-	-

4. Spike Recovery

Spiked Sample Procedure

1. Five (5) neat CSF samples and Ten (10) neat samples (5 serum and 5 K2 EDTA plasma) were spiked with three different concentrations of recombinant analyte, 150 pg/ml, 75.0 pg/mL and 37.5 pg/mL and 0 pg/ml (Standard Diluent). Spiked samples were filtered and then diluted 1:4 for CSF and 1:2 for Serum and Plasma, the Minimum Required Dilutions (MRD) for the assay.

2. Spike recovery is calculated as:

$$\frac{\textbf{interpolated spiked sample} - \textbf{interpolated endogenous}}{\textbf{Nominal Spike}} \times \mathbf{100}\%$$

Spike Recovery Results

Spike recovery ranged between 99% -165% with a mean of 123% for CSF, between 77% -105% with a mean of 93% for serum and between 78% -104% with a mean of 94% for plasma.

CSF Spike Recovery:

Sample ID	n	Spiked [NF-L] pg/mL	Sample Vol (µL/well)	Dilution Corrected Mean [NF-L] pg/mL	SD	CV	Spike Recovery
HMN713784	3	0.0	25	86.22	2.1	2%	-
	3	150.0		234.01	22.2	9%	99%
	3	75.0		184.32	5.1	3%	131%
	3	37.5		146.03	5.7	4%	159%
HMN713785	3	0.0	25	98.59	10.3	10%	-
	3	150.0		251.60	12.9	5%	102%
	3	75.0		183.07	14.0	8%	113%
	3	37.5		147.04	17.0	12%	129%
HMN713786	3	0.0	25	142.11	18.2	13%	-
	3	150.0		308.30	25.4	8%	111%
	3	75.0		227.49	20.9	9%	114%
	3	37.5		199.11	17.5	9%	152%
HMN713787	3	0.0	25	172.99	21.1	12%	-
	3	150.0		346.57	9.7	3%	116%
	3	75.0		253.98	27.6	11%	108%
	3	37.5		235.04	27.5	12%	165%
HMN713788	3	0.0	25	84.32	3.8	4%	-
	3	150.0		254.51	7.1	3%	113%
	3	75.0		172.72	4.8	3%	118%
	3	37.5		130.49	1.0	1%	123%

Serum Spike Recovery:

Sample ID	n	Spiked [NF-L] pg/mL	Sample Vol (µL/well)	Dilution Corrected Mean [NF-L] pg/mL	SD	CV	Spike Recovery
12152255	3	0.0	50	7.90	0.3	4%	-
	3	150.0		163.87	7.9	5%	104%
	3	75.0		71.03	9.9	14%	84%
	3	37.5		45.65	4.3	9%	101%
12152238	3	0.0	50	1.85	0.3	14%	-
	3	150.0		144.08	6.4	4%	95%
	3	75.0		73.79	6.7	9%	96%
	3	37.5		40.62	1.7	4%	103%
12152240	3	0.0	50	5.99	0.8	13%	-
	3	150.0		137.39	5.8	4%	88%
	3	75.0		63.98	5.6	9%	77%
	2	37.5		36.75	2.3	6%	82%
BRH1451736	2	0.0	50	21.89	3.2	15%	-
	3	150.0		180.13	6.4	4%	105%
	3	75.0		87.91	7.7	9%	88%
	3	37.5		56.83	5.0	9%	93%
BRH1451740	2	0.0	50	3.01	0.5	16%	-
	3	150.0		144.66	6.9	5%	94%
	3	75.0		71.42	3.6	5%	91%
	3	37.5		36.19	3.1	8%	88%

Plasma Spike Recovery:

Sample ID	n	Spiked [NF-L] pg/mL	Sample Vol (µL/well)	Dilution Corrected Mean [NF-L] pg/mL	SD	CV	Spike Recovery
2822621	3	0.0	50	3.82	0.2	6%	-
	3	150.0		122.41	5.8	5%	79%
	3	75.0		62.83	8.6	14%	79%
	2	37.5		33.08	4.2	13%	78%
2822620	3	0.0	50	2.49	0.4	16%	-
	3	150.0		144.47	6.7	5%	95%
	3	75.0		76.45	2.2	3%	99%
	3	37.5		38.14	4.7	12%	95%
2822600	3	0.0	50	2.58	0.2	6%	-
	3	150.0		152.26	8.4	6%	100%
	3	75.0		71.12	1.8	2%	91%
	3	37.5		36.96	0.7	2%	92%
HMN108002	3	0.0	50	3.36	0.5	13%	-
	3	150.0		159.29	6.0	4%	104%
	3	75.0		77.53	5.0	6%	99%
	3	37.5		40.69	0.8	2%	100%
HMN108012	2	0.0	50	1.37	0.0	0%	-
	3	150.0		150.42	10.3	7%	99%
	3	75.0		70.30	3.3	5%	92%
	2	37.5		39.70	2.4	6%	102%

5. Parallelism / Dilutional Linearity

Parallelism / Dilutional Linearity Procedure

1. 15 endogenous CSF samples were used to measure parallelism.
   a. 5 healthy donor CSF (CSF)
   b. 5 Multiple sclerosis (MS) donor CSF
   c. 5 Alzheimer's Disease (AD) CSF
   d. The samples were adjusted to MRD (1:4) then serially diluted 2-fold using standard diluent prior to running the assay.
2. 10 samples (5 serum and 5 K2EDTA Plasma) were used to measure dilutional linearity as follows:
   a. 10 samples (5 serum and 5 plasma) were spiked with 150 pg/mL of NF-L standard and then filtered.
   b. The samples were adjusted to MRD (1:2) then serially diluted 2-fold using standard diluent prior to running the assay.

3. Parallelism / Dilution linearity is calculated as follows:

$$\frac{\textbf{Observed concentration}}{\textbf{Expected Concentration}} \times \mathbf{100}\%$$

Where:

1- Observed concentration is the mean calculated dilution-corrected concentration at each dilution.

2- Expected concentration is the mean calculated dilution-corrected concentration at MRD.

Parallelism Results

Parallelism ranged from 85% -114% with an overall average of 99% for 15 normal and diseased samples.

Healthy donor CSF:

Sample ID	n	Dilution Factor	Mean [NF-L] pg/mL	SD	CV	Dilution corrected [NF-L] pg/mL	SD	CV	Parallelism
HMN713784	3	MRD (1:4)	21.35	1.2	6%	85.38	4.7	6%	-
	3	1:8	11.00	0.6	6%	88.04	4.9	6%	103%
	3	1:16	5.86	1.8	30%	93.80	28.4	30%	110%
	3	1:32	2.88	0.3	11%	92.15	10.0	11%	108%
HMN713785	3	MRD (1:4)	24.37	0.6	3%	97.48	2.6	3%	-
	3	1:8	11.65	0.3	2%	93.18	2.3	2%	96%
	3	1:16	5.86	0.7	12%	93.83	11.2	12%	96%
	3	1:32	3.46	0.3	8%	110.72	8.4	8%	114%
HMN713786	3	MRD (1:4)	35.58	1.0	3%	142.31	4.2	3%	-
	3	1:8	16.42	0.9	5%	131.35	6.9	5%	92%
	3	1:16	8.17	0.4	5%	130.65	6.1	5%	92%
	3	1:32	4.69	0.2	4%	150.01	5.8	4%	105%
HMN713787	2	MRD (1:4)	45.07	4.9	11%	180.27	19.7	11%	-
	3	1:8	20.18	2.1	11%	161.45	17.1	11%	90%
	3	1:16	9.88	0.7	8%	158.15	12.0	8%	88%
	3	1:32	5.75	0.9	16%	184.09	29.3	16%	102%
HMN713788	3	MRD (1:4)	21.56	3.2	15%	86.25	12.6	15%	-
	3	1:8	11.27	0.4	4%	90.12	3.3	4%	104%
	3	1:16	5.95	1.1	19%	95.17	17.9	19%	110%
	3	1:32	2.85	0.1	5%	91.35	4.2	5%	106%

Multiple Sclerosis CSF:

Sample ID	n	Dilution Factor	Mean [NF-L] pg/mL	SD	CV	Dilution corrected [NF-L] pg/mL	SD	CV	Parallelism
HMN686704	3	MRD (1:4)	17.73	0.7	4%	70.91	2.7	4%	-
	3	1:8	8.46	1.2	14%	67.70	9.5	14%	95%
	3	1:16	4.26	0.7	16%	68.22	11.0	16%	96%
	2	1:32	2.10	0.4	19%	67.04	12.8	19%	95%
HMN686703	3	MRD (1:4)	17.01	1.5	9%	68.03	6.0	9%	-
	3	1:8	8.08	0.4	4%	64.62	2.9	4%	95%
	3	1:16	4.48	0.3	7%	71.65	5.1	7%	105%
	3	1:32	2.43	0.3	14%	77.78	10.6	14%	114%
HMN686702	3	MRD (1:4)	34.76	4.3	12%	139.04	17.4	12%	-
	3	1:8	14.78	0.9	6%	118.22	7.3	6%	85%
	3	1:16	7.38	0.7	10%	118.06	11.5	10%	85%
	3	1:32	4.15	0.4	10%	132.84	13.6	10%	96%
HMN686705	3	MRD (1:4)	20.60	1.1	5%	82.41	4.2	5%	-
	3	1:8	10.47	1.0	10%	83.78	8.1	10%	102%
	3	1:16	4.36	1.2	29%	69.83	20.0	29%	85%
	2	1:32	2.82	0.6	21%	90.30	19.4	21%	110%
HMN686707	3	MRD (1:4)	26.71	1.5	6%	106.83	5.9	6%	-
	3	1:8	13.33	0.9	7%	106.61	7.0	7%	100%
	3	1:16	6.87	0.4	5%	109.98	5.8	5%	103%
	2	1:32	3.37	0.3	9%	107.84	10.2	9%	101%

Alzheimer's Disease CSF:

Sample ID	n	Dilution Condition	Mean [NF-L] pg/mL	SD	CV	Dilution corrected [NF-L] pg/mL	SD	CV	Parallelism
HMN629578	3	MRD (1:4)	46.58	6.1	13%	186.33	24.3	13%	-
	3	1:8	22.63	4.4	20%	181.07	35.6	20%	97%
	3	1:16	11.05	1.4	13%	176.82	22.2	13%	95%
	3	1:32	5.46	0.4	7%	174.65	11.7	7%	94%
HMN629510	3	MRD (1:4)	806.11	33.8	4%	3224.42	135.2	4%	-
	3	1:8	395.42	19.9	5%	3163.37	159.4	5%	98%
	3	1:16	181.07	21.1	12%	2897.10	336.8	12%	90%
	3	1:32	92.14	3.6	4%	2948.33	115.0	4%	91%
HMN627647	3	MRD (1:4)	31.56	2.8	9%	126.24	11.4	9%	-
	3	1:8	17.72	1.3	7%	141.78	10.1	7%	112%
	3	1:16	8.32	0.6	7%	133.17	9.4	7%	105%
	2	1:32	4.08	0.6	14%	130.70	18.1	14%	104%
HMN686180	3	MRD (1:4)	74.93	1.3	2%	299.71	5.4	2%	-
	3	1:8	36.40	4.0	11%	291.17	32.0	11%	97%
	3	1:16	18.67	0.5	3%	298.79	7.7	3%	100%
	3	1:32	8.53	0.8	10%	272.96	26.4	10%	91%
HMN641507	3	MRD (1:4)	27.58	2.0	7%	110.33	8.0	7%	-
	3	1:8	14.52	1.0	7%	116.13	7.9	7%	105%
	3	1:16	6.72	0.2	3%	107.54	2.8	3%	97%
	3	1:32	3.50	0.2	5%	112.04	6.0	5%	102%

Dilutional Linearity Results

The linearity ranged from 90%-115% with an overall average of 106% for plasma samples. The linearity ranged from 98% - 119% with an overall average of 110% for serum samples.

Serum Linearity:

Sample ID	n	Dilution Factor	Mean [NF-L] pg/mL	SD	CV	Dilution corrected [NF-L] pg/mL	SD	CV	Dilutional Linearity
12152255	3	MRD (1:2)	61.41	4.9	8%	122.81	9.9	8%	-
	3	1:4	31.80	1.2	4%	127.21	4.7	4%	104%
	3	1:8	16.01	0.1	1%	128.05	1.0	1%	104%
	3	1:16	7.56	0.5	6%	120.89	7.5	6%	98%
12152238	3	MRD (1:2)	58.48	6.0	10%	116.96	12.0	10%	-
	3	1:4	31.18	0.6	2%	124.70	2.4	2%	107%
	3	1:8	15.90	1.1	7%	127.21	9.0	7%	109%
	3	1:16	7.60	0.1	2%	121.65	2.0	2%	104%
12152240	3	MRD (1:2)	49.81	3.2	6%	99.61	6.4	6%	-
	3	1:4	28.51	1.0	3%	114.04	4.0	3%	114%
	3	1:8	14.85	0.2	1%	118.80	1.5	1%	119%
	3	1:16	6.97	0.7	10%	111.53	10.9	10%	112%
BRH1451736	3	MRD (1:2)	68.10	3.9	6%	136.20	7.7	6%	-
	3	1:4	37.37	0.6	2%	149.49	2.5	2%	110%
	3	1:8	19.82	0.6	3%	158.53	4.6	3%	116%
	3	1:16	9.33	0.6	6%	149.30	9.2	6%	110%
BRH1451740	3	MRD (1:2)	54.72	1.5	3%	109.45	3.0	3%	-
	3	1:4	29.09	0.4	1%	116.37	1.4	1%	106%
	3	1:8	15.88	1.8	11%	127.07	14.3	11%	116%
	3	1:16	7.74	0.4	5%	123.77	5.7	5%	113%

Plasma Linearity:

Sample ID	n	Dilution Factor	Mean [NF-L] pg/mL	SD	CV	Dilution corrected [NF-L] pg/mL	SD	CV	Dilutional Linearity
2822621	3	MRD (1:2)	56.40	1.0	2%	112.80	1.0	1%	-
	3	1:4	31.71	1.1	3%	126.84	1.1	1%	112%
	3	1:8	15.82	1.0	6%	126.59	1.0	1%	112%
	3	1:16	8.04	0.1	2%	128.61	0.1	0%	114%
2822620	3	MRD (1:2)	65.30	1.4	2%	130.59	1.4	1%	-
	3	1:4	31.75	1.1	4%	126.99	1.1	1%	97%
	3	1:8	16.42	1.2	7%	131.38	1.2	1%	101%
	3	1:16	7.33	0.1	2%	117.31	0.1	0%	90%
2822600	3	MRD (1:2)	64.20	6.5	10%	128.41	6.5	5%	-
	3	1:4	31.31	0.7	2%	125.23	0.7	1%	98%
	3	1:8	17.04	0.1	1%	136.34	0.1	0%	106%
	3	1:16	7.85	0.3	4%	125.62	0.3	0%	98%
HMN108002	3	MRD (1:2)	58.66	4.7	8%	117.32	4.7	4%	-
	3	1:4	33.87	1.0	3%	135.47	1.0	1%	115%
	3	1:8	15.47	0.2	2%	123.78	0.2	0%	106%
	3	1:16	8.41	0.9	11%	134.54	0.9	1%	115%
HMN108012	3	MRD (1:2)	57.71	7.9	14%	115.42	7.9	7%	-
	3	1:4	29.56	1.5	5%	118.24	1.5	1%	102%
	3	1:8	15.55	1.0	6%	124.38	1.0	1%	108%
	3	1:16	8.14	0.6	7%	130.23	0.6	0%	113%

6. Precision

Assay Precision Procedure

To assess intra- and inter-assay precision of the NF-L assay, five normal plasma samples were run in triplicate over 3 days by 3 different operators on 1 plate per day.

Assay Precision Results

		Intra-assay			Inter-assay		
Sample	**n**	**Mean [NF-L] pg/mL**	**SD**	**CV**	**Mean [NF-L] pg/mL**	**SD**	**CV**
12319408	3	3.98	0.4	9%	3.72	0.5	14%
	2	4.04	0.5	12%			
	2	3.14	0.2	5%			
12319407	3	2.92	0.4	13%	2.85	0.4	13%
	3	2.44	0.4	15%			
	2	3.20	0.3	10%			
12310014	3	4.70	0.7	15%	4.56	0.4	9%
	3	4.10	0.5	13%			
	3	4.88	0.8	15%			
HMN108000	3	8.00	0.7	8%	7.83	0.6	8%
	3	7.14	0.7	10%			
	3	8.36	0.5	6%			
HMN108005	3	6.48	0.4	6%	6.47	0.3	4%
	3	6.20	1.2	19%			
	2	6.74	0.2	3%			

7. Results for Serum & Plasma (or other Matrix if tested)

- This assay has been verified in human serum, plasma, and CSF.
- No other matrices have been tested.

8. Results for Known Diseased Samples

- Alzheimer's Disease (AD)
  - CSF Endogenous Levels 64.62 – 132.84 pg/mL (Mean 90.30 pg/mL)
  - CSF Parallelism 90% – 112% (Mean 99%)
- Multiple Sclerosis (MS)
  - CSF Endogenous Levels 107.54 – 3163.37 pg/mL (Mean 743.04 pg/mL)
  - CSF Parallelism 85% – 114% (Mean 98%)

9. Analyte Cross-Reactivity / Interference Testing

- This assay was tested for cross reactivity with NF-M, NF-H, pTau181, tTau, and GFAP
  - There is no cross-reactivity detected with these analytes.

10. Assay Format

- Capture: Monoclonal

- Detection: Monoclonal
- Analyte: Native Protein