

Human NF-L

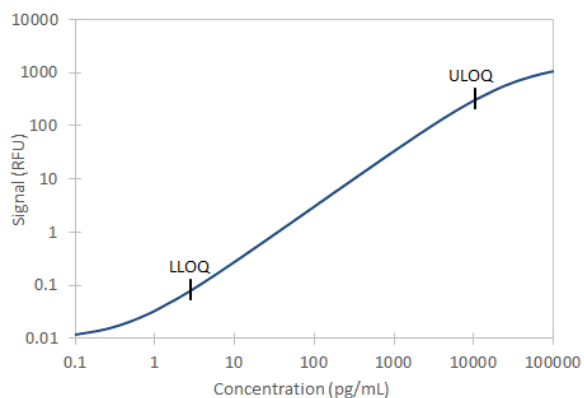
Product Description

Simple Plex™ assay is for the detection of human Neurofilament Light (NF-L) in cell culture supernatant (CCS), serum, plasma (EDTA/Heparin), and cerebrospinal fluid (CSF) in Sample Diluent SD13.

Calibration Curve

The factory generated calibration curve shown below was compiled by averaging replicates of each calibrator from multiple runs. The 4PL curve fit shows calibrator concentration as a function of signal intensity (relative fluorescent units, RFU).

Human serum and plasma assays are quality control tested using a serum/plasma based Bio-Techne Reference Material. Curves may be adjusted to this material for lot-to-lot consistency.



Limits of Quantification

Data shown represents typical performance results of the Human NF-L Simple Plex Assay.

	Concentration (pg/mL)
Limit of Detection (LOD)	1.09
Lower Limit of Quantitation (LLOQ)	2.70
Upper limit of Quantitation (ULOQ)	10,290

LOD is calculated by adding three standard deviations to the mean background signal determined from multiple runs.

LLOQ and ULOQ are calculated across multiple cartridge lots and Ellas as the in-well concentration range in which curve points recover 80-120% with a coefficient of variation (CV) of less than 20%, and in which measured samples recover 80-120% upon serial dilution with a CV less than 15%.

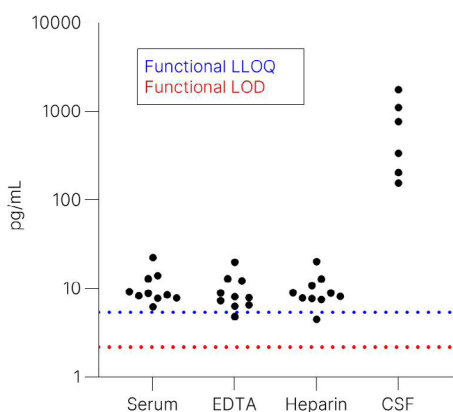
Endogenous Levels

Endogenous levels were calculated from multiple samples. Samples were from apparently healthy volunteers. No medical histories were available for the donors in this study. The values provided below are for informational purposes only. Actual concentration values and detectability rates may vary across different sample cohorts.

Sample Type	Mean* (pg/mL)	Median (pg/mL)	% above LLOQ	% above LOD
Serum (n=10)	10.6	8.7	100	100
EDTA plasma (n=10)	10.0	8.1	90	100
Heparin plasma (n=10)	10.3	9.0	90	100
CSF (n=6)	721	551	100	100

*Values below LLOQ are excluded from the mean.

Endogenous Levels *continued*



Functional LOD and functional LLOQ are calculated as the LOD and LLOQ of the assay multiplied by the minimum required dilution (MRD) for the sample type.

Precision

Intra-Assay Precision: Multiple replicates of each control were tested in one assay.

Inter-Assay Precision: Replicates of each control were tested in multiple runs performed by at least three technicians using two lots of reagents.

Parameter	Intra-Assay		Inter-Assay	
	Low	High	Low	High
n	16	16	48	48
Mean (pg/mL)	76.6	3309	80.5	3476
Standard Deviation	3.64	154	8.35	267
CV (%)	4.8	4.7	10.4	7.7

Recovery

Recovery at three different spiked concentrations within the range of the assay was evaluated.

Sample Type	Average%	Range%
CCS (n=5)	112	110-115
Serum (n=4)	108	95-115
EDTA plasma (n=4)	93	86-99
Heparin plasma (n=4)	101	93-106

Linearity

Samples containing and/or spiked with high concentrations of human NF-L were serially diluted with Sample Diluent to produce samples within the dynamic range of the assay.

Dilution	Parameter	CCS (n=5)	Serum (n=4)	EDTA Plasma (n=4)	Heparin Plasma (n=4)	CSF (n=4)
1:2	Avg % of Expected	85	96	102	100	94
	Range (%)	84-86	92-102	98-108	96-103	90-97
1:4	Avg % of Expected	80	94	103	99	90
	Range (%)	78-82	89-103	99-110	94-105	88-93
1:8	Avg % of Expected	80	95	106	102	91
	Range (%)	79-82	88-106	100-119	94-110	88-94
1:16	Avg % of Expected	84	98	111	107	90
	Range (%)	80-88	90-110	105-118	101-116	80-95

Specificity

This assay recognizes natural and recombinant human NF-L. The factors listed were prepared at 900 ng/mL in Sample Diluent and assayed for cross-reactivity. Preparations of the following factors at 900 ng/mL in a recombinant human NF-L control were assayed for interference. No significant cross-reactivity or interference was observed.

Recombinant human:

- 14-3-3 β
- 14-3-3 η
- 14-3-3 θ
- 14-3-3 ζ
- Contactin-2

Natural proteins:

- NF-H
- NF-M

Sample Preparation

CCS samples require a minimum 5-fold dilution with sample diluent. A suggested 5-fold dilution can be achieved by adding 20 μ L of sample to 80 μ L of Sample Diluent. Samples above the ULOQ require further dilution.

Serum, plasma, and CSF samples require a minimum 2-fold dilution with sample diluent. A suggested 2-fold dilution can be achieved by adding 35 μ L of sample to 35 μ L of Sample Diluent. Samples above the ULOQ require further dilution.

Assay Protocol

Refer to the Product Insert and CoA provided on our website for the specific assay protocol and precautions. A generic product protocol can be found online at: www.bio-techne.com/simple-plex-protocol

Download the CoA and product insert:
www.bio-techne.com/resources/cofa-finder-tool

Sample Collection and Storage

The sample collection and storage conditions listed below are intended as general guidelines. Sample stability has not been evaluated.

Cell Culture Supernatant: Remove particulates by centrifugation and assay immediately or aliquot and store samples at ≤ -20 °C. Avoid repeated freeze-thaw cycles.

Serum: Use a serum separator tube (SST) and allow samples to clot for 30 minutes at room temperature before centrifugation for 15 minutes at 1000 x g. Remove serum and assay immediately or aliquot and store samples at ≤ -20 °C. Avoid repeated freeze-thaw cycles.

Plasma: Collect plasma using EDTA or heparin as an anticoagulant. Centrifuge for 15 minutes at 1000 x g within 30 minutes of collection. Assay immediately or aliquot and store samples at ≤ -20 °C. Avoid repeated freeze-thaw cycles.

Cerebrospinal Fluid: Freeze sample within one hour of collection to avoid aggregation. Store samples at ≤ -70 °C.

Note: Grossly hemolyzed or icteric samples are not suitable for use with this assay.