NF-light™ Serum ELISA

20-8002 RUO

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Neurofilaments are the main cytoskeletal constituents in neuronal cells. They are important for the maintenance of axonal caliber and morphological integrity, which affect the velocity and fidelity of neuronal transmissions. Three different neurofilament chains exist and are named according to their size: Neurofilament light, medium and heavy. Neurofilament light constitutes the backbone to which the heavier chains co-assemble to form the neurofilament fiber. Following injuries of nerve cells due to direct trauma or slow degenerative processes, the content of the cell is released into the surrounding compartment and allows quantitative determinations of the axonal proteins[1,2].

Intended Use

Serum NF-light™ ELISA is a Research Use Only assay for quantitative measurements of human Neurofilament light (NF-L) protein in serum samples. The kit is intended for professional use.

Method Description

The UmanDiagnostics NF-light™ Serum ELISA assay is an enzymatic immunoassay using two highly specific, non-competing, monoclonal antibodies[3]. The capture antibody is coated on a solid surface and binds the sample NF-L. The secondary / detection antibody is biotin conjugated and addition of HRP-conjugated Streptavidin allows for quantitative determinations by enzymatic turn-over of a colorless substrate (TMB) to a colored product. The absorbance value can be correlated to the amount of NF-L in the sample by using the calibrator curve.

Calibrator Curve Range	0.5 - 40 pg/mL
Max. Sample Size*	50 µL / replicate
Diluted Sample Volume	200 µL / replicate
Min. Dilution of Sample	4x
Total Incubation Time	4 hours & 15 minutes

* When sample is diluted 4 times.

Sensitivity

LLoD	0.4 pg/mL
LLoQ	0.8 pg/mL

The calculations were based on the method described in the NCCLS definition EP17-4

Calibrator Curve

Four Parameter Marquardt (Automatic weighting using relative weights (1/Y2))

1. Yuan A., et al., Neurofilaments and Neurofilament Proteins in Health and Disease. Cold Spring Harb Perspect Biol, 2017. 9(4).
2. Gaetani L., et al., Neurofilament light chain as biomarker in neurological disorders. J Neurol Neurosurg Psychiatry, 2019;90:870-881.
3. Norgren N, Karlsson JE, Rosengren L, Stigbrand T. Monoclonal antibodies selective for low molecular weight neurofilaments. Hybrid Hybridomics. 2002 Feb; 21(1): 53-

Precision

Inter-precision

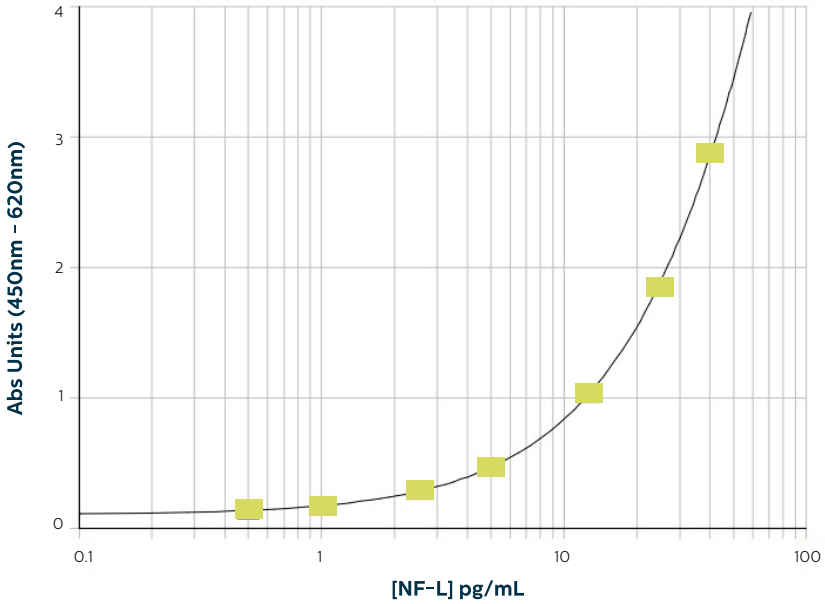
10 serum samples ranging from 10 to 130 pg/mL were analyzed in duplicate on 9 or 10 separate occasions. The CV (%) for each sample was calculated (n= 9 or 10) and the inter-precision was reported as the mean CV (%) of its respective concentration range.

Inter-precision (10-15 pg/mL)	10% (8.7 - 11.2)
Inter-precision (15-130 pg/mL)	5.7% (3.9 - 6.8)

Intra-precision

4 serum samples ranging from 5 to 125 pg/mL were analyzed in six replicates at three different occasions. The CV (%) for each sample was calculated for each separate occasion (n=6) and the intra-precision is reported as the mean CV (%) of its respective concentration range.

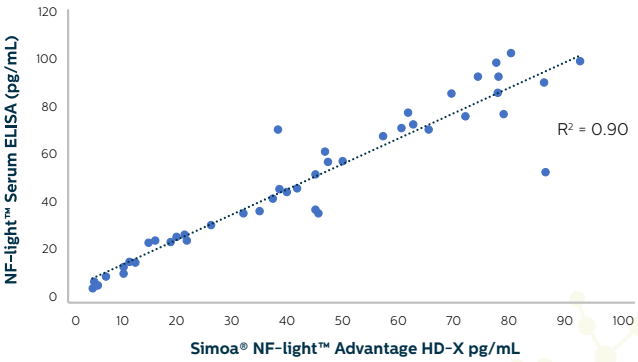
Intra-precision (5-10 pg/mL)	6.6% (4.2 - 11.1)
Intra-precision (10-125 pg/mL)	4.3% (2.9 - 5.9)



Correlation to Simoa® NF-light™

45 unique serum samples were measured at two different sites by either the NF-light™ Serum ELISA or the Simoa® NF-light™ Advantage assay. Each sample was measured in duplicates at two occasions and the mean read-outs from each method were plotted against each other.

The correlation of the NF-light™ Serum ELISA to the Simoa® NF-light™ Advantage is high, R2 = 0.90, and the ELISA has a mean read-out level 27% higher than the Simoa.



Dilution Linearity

Six serum samples were spiked with endogenous NF-L and serially diluted 4 - 128 times. The read-out (pg/mL) for each measurement was multiplied by its respective dilution factor and the mean concentration of each sample was calculated from all values within the calibrator curve quantification range. Linearity was calculated for each measurement by relating it to its respective mean.

Linearity (4x)	98% (89 - 103%)
Linearity (32x)	103% (96 - 109%)
Linearity (128x)	89% (84 - 101%)

Parallelism

Eight serum samples with high endogenous levels of NF-L were 2-fold serially diluted, three times, from an original 4-time dilution. Each measurement was multiplied by its respective dilution factor and the CV (%) for each sample was calculated (n=4). Parallelism was defined as sample CV:s below 20%.

Parallelism (CV < 20%)	Mean = 9.2%
	Range: 6.3 - 13.5%

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