SPEAR UltraDetect™ Mouse NF-L

Item SPR90037 Datasheet

Description

SPEAR UltraDetect™ Mouse NF-L was analytically verified for use in mouse K2EDTA plasma specimens.*

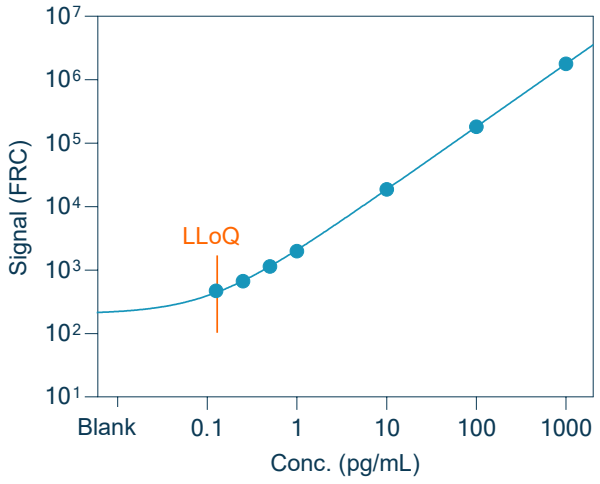
Assay Sensitivity and Range

Lower limit of detection (LLoD) was calculated as 2.5x standard deviations added to the mean blank. Lower limit of quantification (LLoQ) was calculated as lowest concentration with mean CV under 20% and 80-120% recovery. Results were generated from a minimum of 6 independent runs.

Specification	Result
Analytical LLoD (range)	0.037 pg/mL (0.019 – 0.070)
Functional LLoD	0.293 pg/mL
Analytical LLoQ	0.125 pg/mL
Functional LLoQ	1.00 pg/mL
Minimum required dilution (MRD)‡	8x
Functional Assay Range	1.00 – 8000 pg/mL

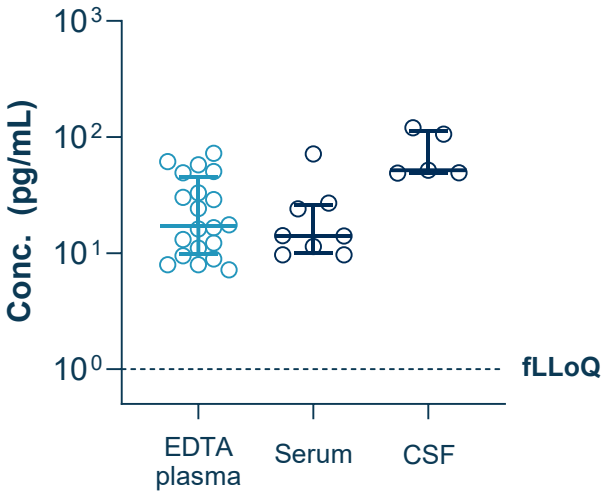
Calibration Curve

Representative calibration curve fitted with 4PL $1/y^2$ weighting. Analytical LLoQ is indicated in orange.



Endogenous Levels

Commercially sourced EDTA plasma, serum†, and CSF† samples from young mice were tested. All samples were diluted 8x. Medians with interquartile ranges are shown for each specimen type with functional LLoQ depicted as dashed horizontal line.



Sample Type (donors)	Median Conc. (range)	Above LLoD %	Above LLoQ %
EDTA Plasma (n=20)	17.0 pg/mL (7.19 – 72.3)	100%	100%
Serum† (n=8)	14.0 pg/mL (9.7 – 71.4)	100%	100%
CSF† (n=5)	51.7 pg/mL (49.0 – 120)	100%	100%

*All data shown were generated in 384-well runs with F.A.S.T. workflow and qPCR amplification on QuantStudio instruments.

‡ All F.A.S.T. workflows include a 4x MRD. This assay requires an additional 2x pre-workflow dilution for a total of 8x MRD. Users may apply alternative pre-workflow dilutions as determined appropriate for their samples. Endogenous mouse NF-L is stable in both neat EDTA plasma and EDTA plasma diluted 1:2 in assay diluent when stored for up to 4 hours at room temperature or up to 18 hours at 4°C.

†Endogenous levels of NF-L in mouse serum and CSF were tested for reference only. Full verification of assay performance is recommended before using these matrices in studies.

Precision

Pooled mouse EDTA plasma samples with or without calibrator spike in representing 3 different analyte concentrations, were run in duplicates per run across 6 runs to assess intra- and inter-assay precision.

Sample Type	Mean Conc. pg/mL	Mean Intra-assay CV (range)	Inter-assay CV
EDTA Plasma	30.4	1.0% (0.0 – 2.7%)	10.1%
	138	1.4% (0.4 – 2.4%)	8.5%
	1081	1.0% (0.4 – 1.7%)	7.8%

Spike Recovery

To assess recovery in native matrix, mouse EDTA plasma samples (n = 3) were spiked with calibrator at low, medium, and high concentrations (respective to endogenous levels and assay dynamic range) and diluted at MRD. Percent recovery was calculated as the endogenous-subtracted spiked sample concentration divided by the expected concentration of the spike material.

Sample Type (donors)	Spike Conc.	Mean Recovery (Range)	Recovery
EDTA Plasma (n=3)	Low	101% (92.9 – 110%)	93.4%
	Med	87.1% (84.2 – 90.0%)	
	High	92.3% (89.5 – 95.6%)	

Dilution Linearity

Mouse EDTA plasma samples (n = 3) were spiked with calibrator and serially diluted 2x to 32x above assay MRD. Percent recovery was calculated as the sample concentration at higher dilution divided by the sample concentration at MRD.

Sample Type (donors)	Dilution	Mean Recovery (Range)	Recovery
EDTA Plasma (n=3)	2x	103% (103 - 104%)	101%
	4x	102% (99.8 – 105%)	
	8x	101% (101 – 102%)	
	16x	99.3% (98.4 – 100%)	
	32x	97.5% (94.8 – 102%)	

Interference

Potential interference was evaluated at low, medium, and high biologically relevant levels for hemoglobin (Hb), triglycerides (TG), conjugated bilirubin (CB), and unconjugated bilirubin (UB). Table below shows the highest concentration at which no significant interference was observed with percent difference from vehicle reported.

Potential Interferent & Conc.		EDTA plasma % diff.
Hb	High conc. 400 mg/dL	2.9%
TG	High conc. 500 mg/dL	-7.5%
CB	High conc. 2.5 mg/dL	-5.0%
UB	High conc. 2.5 mg/dL	-6.3%

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