SPEAR UltraDetect™ GFAP

Item SPR90010 Datasheet

Description

SPEAR UltraDetect™ GFAP (Glial Fibrillary Acidic Protein) was analytically verified for use with human EDTA plasma and serum specimens.**

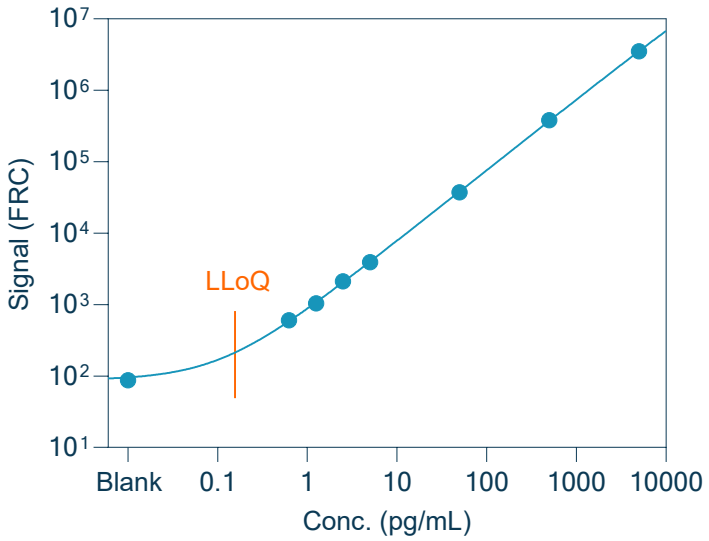
Assay Sensitivity and Range

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

Specification	Result
Analytical LLoD (range)	0.041 pg/mL (0.015-0.067)
Functional LLoD	0.163 pg/mL
Analytical LLoQ	0.156 pg/mL
Functional LLoQ	0.625 pg/mL
Minimum required dilution (MRD)	4x (EDTA plasma, serum)
Functional Assay Range	0.625 – 20,000 pg/mL

Calibration Curve

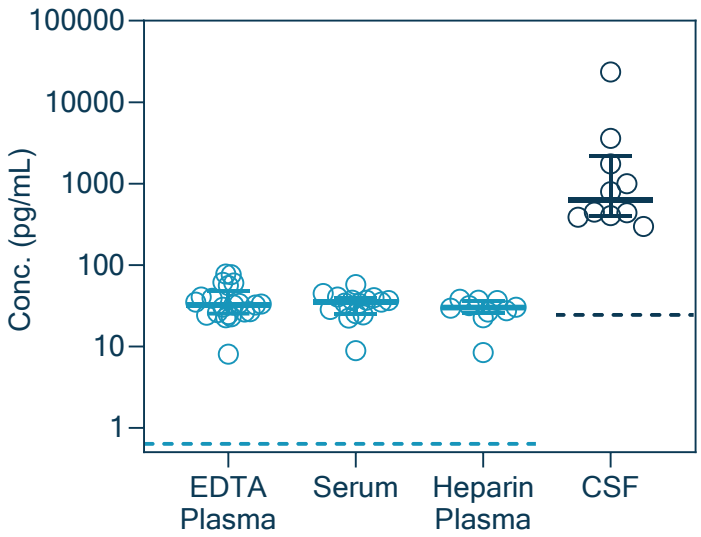
Representative calibration curve fitted with 4PL $1/y^2$ weighting. Analytical LLoQ is indicated in orange.



**All data shown were generated in 96-well runs with F.A.S.T. workflow and qPCR amplification on QuantStudio instruments.

Endogenous Levels

Human EDTA plasma, serum, heparin plasma*, and CSF* from apparently healthy donors were tested. Plasma and serum samples were diluted 4x and CSF 100x. Medians with interquartile ranges are shown for each specimen type with functional LLoQ indicated as dashed horizontal line.



Sample Type (donors)	Mean Conc. (range)	Above LLoD %	Above LLoQ %
EDTA plasma (21 donors)	37.5 pg/mL (8.0-77.2)	100%	100%
Serum (15 donors)	33.6 pg/mL (8.9-57.5)	100%	100%
Heparin plasma* (10 donors)	28.8 pg/mL (8.4-37.8)	100%	100%
CSF* (10 donors)	3263 pg/mL (299-23535)	100%	100%

*Endogenous levels for heparin plasma and CSF were tested for reference only. Full verification of assay performance is recommended before using these matrices in studies.

Benchmark

EDTA Plasma samples (n=37) with concentrations spanning 1.6 logs were measured on SPEAR UltraDetect GFAP and a commercial singleplex GFAP immunoassay, demonstrating a linear correlation with R^2 of 0.99.

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Spike Recovery

To assess the selectivity of the assay, EDTA Plasma and Serum from apparently healthy donors (n = 3) were spiked with CSF 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 measured concentration of the spike material.

Sample Type (donors)	Spike Conc.	Mean Recovery (range)	Recovery
EDTA Plasma (3 donors)	Low	107% (100-117%)	106%
	Med	103% (96-108%)	
	High	108% (100-114%)	
Serum (3 donors)	Low	103% (100-107%)	103%
	Med	102% (98-108%)	
	High	103% (99-108%)	

Dilution Linearity

To assess the accuracy of the assay, EDTA Plasma and Serum from apparently healthy donors (n = 3) were spiked with CSF 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 (3 donors)	2x	98% (95-103%)	94%
	4x	96% (92-102%)	
	8x	96% (91-105%)	
	16x	90% (85-98%)	
	32x	90% (84-99%)	
Serum (3 donors)	2x	99% (97-100%)	92%
	4x	94% (93-96%)	
	8x	92% (90-95%)	
	16x	89% (86-91%)	
	32x	87% (86-88%)	

Precision

EDTA Plasma and Serum, unspiked or spiked with CSF, representing low, medium, and high analyte concentrations were run in duplicates per run across a minimum of 6 runs to assess intra- and inter-assay precision.

Sample Type	Conc. pg/mL	Mean Intra-assay CV (range)	Inter-assay CV
EDTA Plasma	55.2	1.1% (0.02-3.0%)	4.9%
	252	1.2% (0.2-2.5%)	3.9%
	3311	1.8% (0.2-5.2%)	5.5%
Serum	31.3	2.8% (0.6-6.7%)	6.4%
	332	1.0% (0.1-3.0%)	4.7%
	1535	2.2% (0.9-5.2%)	5.7%

Specificity & Interference

Similar proteins were evaluated for cross-reactivity at 25x the top calibrator concentration and potential interference at 2x endogenous levels. No significant cross-reactivity or interference was observed for:

- Desmin protein

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	-18.0%
TG	High conc. 500 mg/dL	-0.96%
CB	High conc. 2.5 mg/dL	0.18%
UB	High conc. 2.5 mg/dL	-1.29%

Potential Interferent & Conc.		Serum % diff.
Hb	High conc. 400 mg/dL	-10.2%
TG	High conc. 500 mg/dL	-14.6%
CB	High conc. 2.5 mg/dL	-1.32%
UB	High conc. 2.5 mg/dL	-4.38%

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