SPEAR UltraDetect™ pTau 231

Item SPR90009 Datasheet

Description

SPEAR UltraDetect™ pTau 231 (Tau phosphorylated at threonine 231) was analytically verified for use with human EDTA plasma specimens.**

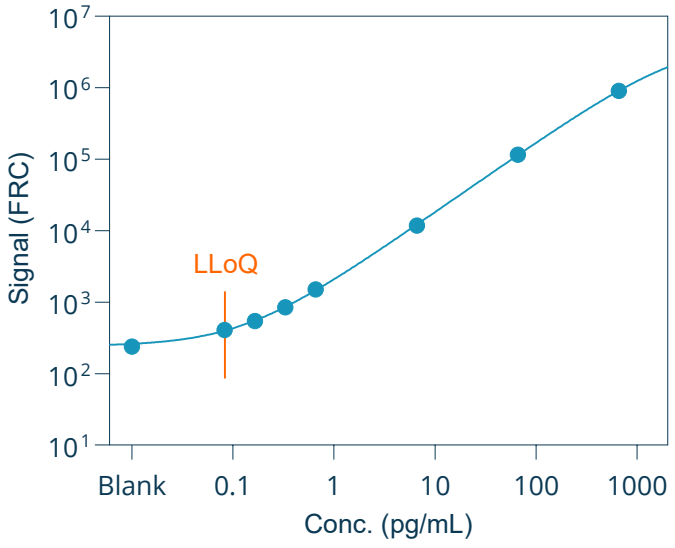
Assay Sensitivity and Range

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% and 80-120% recovery. Results were generated from a minimum of 6 independent runs.

Specification	Result
Analytical LLoD (range)	0.039 pg/mL (0.022-0.106)
Functional LLoD	0.157 pg/mL
Analytical LLoQ	0.083 pg/mL
Functional LLoQ	0.330 pg/mL
Minimum required dilution (MRD)	4x (EDTA plasma)
Functional Assay Range	0.330 – 2640 pg/mL

Calibration Curve

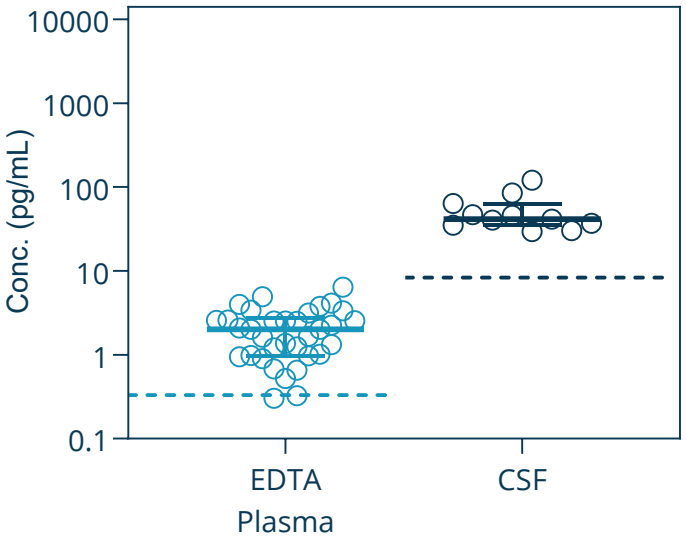
Representative calibration curve fitted with 4PL $1/y^2$ weighting. Analytical LLoQ is indicated in orange.



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



Sample Type (donors)	Mean Conc. (range)	Above LLoD %	Above LLoQ %
EDTA plasma (34 donors)	2.13 pg/mL (0.304-6.41)	100%	97%
CSF* (11 donors)	52.2 pg/mL (29.3-120)	100%	100%

*Endogenous levels for CSF were tested for reference only. Full verification of assay performance is recommended before using this matrix in studies.

Benchmark

EDTA Plasma samples (n=40) with concentrations spanning 1.5 logs were tested on SPEAR UltraDetect pTau 231 and a commercial immunoassay, demonstrating a linear correlation with R^2 of 0.88.

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Precision

Unspiked EDTA Plasma or EDTA Plasma spiked with CSF, representing six different 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	2.20	4.9% (2.4-10.4%)	8.2%
	4.95	2.6% (0.01-5.8%)	5.8%
	9.45	2.9% (0.4-7.0%)	7.2%
	18.2	1.9% (0.2-5.5%)	6.5%
	36.4	2.0% (1.1-3.3%)	4.8%
	68.4	2.6% (1.1-4.5%)	6.3%

Spike Recovery

To assess the selectivity of the assay, EDTA Plasma samples 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	94% (79-104%)	103%
	Med	106% (104-110%)	
	High	108% (105-111%)	

Dilution Linearity

To assess the accuracy of the assay, EDTA Plasma from apparently healthy donors (n = 3) was 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	93% (91-95%)	89%
	4x	88% (87-90%)	
	8x	89% (86-90%)	
	16x	88% (86-90%)	
	32x	89% (88-90%)	

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:

- Total Tau
- Tau phosphorylated at threonine 181
- Tau phosphorylated at threonine 217

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*	Med conc. 200 mg/dL	-10.6%
TG	High conc. 500 mg/dL	-9.8%
CB	High conc. 2.5 mg/dL	-9.8%
UB	High conc. 2.5 mg/dL	-3.8%

*Measurement of pTau 231 in grossly hemolyzed samples containing greater than 200 mg/dL hemoglobin is not recommended.

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