

Description

SPEAR TrueDetect™ eMTBR-Tau (endogenously cleaved microtubule binding region-containing Tau) was analytically verified^a for use in human K2EDTA plasma.

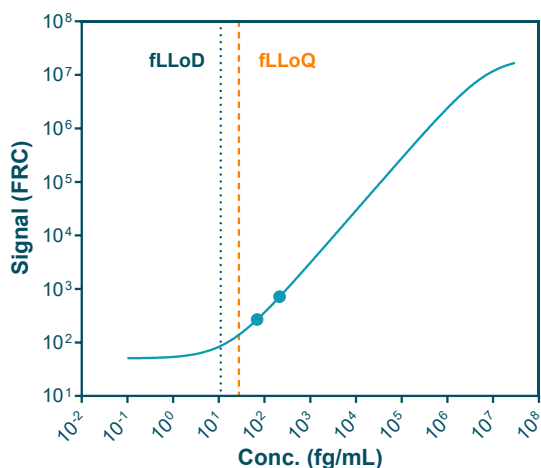
Assay Sensitivity and Range

Lower limit of detection (LLoD) was calculated as 2.5x standard deviations added to the mean signal of blank. Lower limit of quantification (LLoQ) was calculated as the lowest concentration with 75-125% recovery and $\leq 25\%$ CV. Results were generated from a minimum of 6 independent runs.

Specification	Result
LLoD ^b	2.76 fg/mL
Functional LLoD (Range)	11.0 fg/mL (2.65 – 19.1)
LLoQ ^b	6.88 fg/mL
Functional LLoQ	27.5 fg/mL
Minimum required dilution (MRD)	4x
Functional Assay Range	27.5 – 440,000 fg/mL

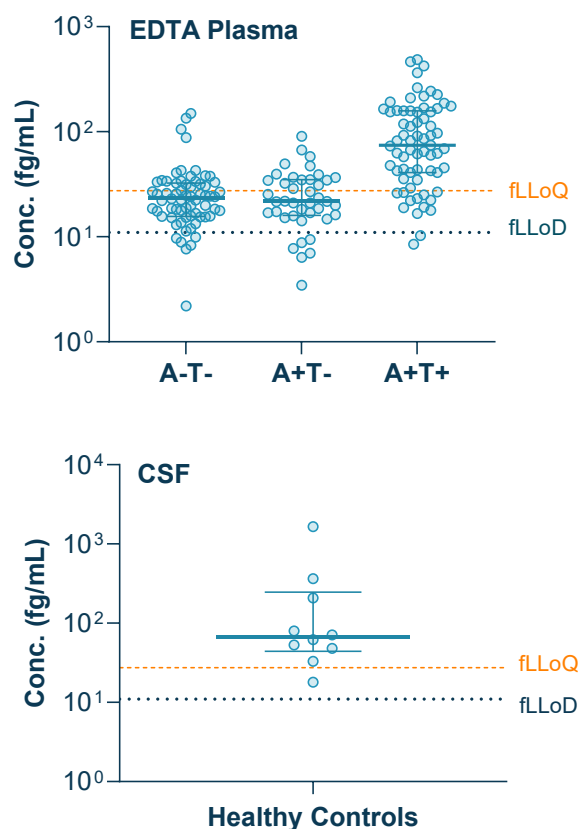
Master Calibration Curve

Representative 2-point adjusted master calibration curve fitted with 4PL $1/y^2$ weighting with functional LLoD (fLLoD) and functional LLoQ (fLLoQ) indicated.



Endogenous Levels

Human EDTA plasma samples^c from amyloid (A) and Tau (T) PET-confirmed donors (upper) and CSF samples from apparently healthy donors (lower) were tested. Medians with interquartile ranges are shown as solid lines.



Sample Type (donors)	Mean Conc. (range)	Above LLoD %	Above LLoQ %
EDTA Plasma A-T- (66 donors)	28.6 fg/mL (2.20 - 150)	90.9%	33.3%
EDTA Plasma A+T- (41 donors)	26.9 fg/mL (3.46 - 90.5)	85.4%	39.0%
EDTA Plasma A+T+ (67 donors)	113 fg/mL (8.48 - 484)	97.0%	80.6%
CSF ^d (10 donors)	258.7 fg/mL (18.3 - 1649)	100%	90.0%

Precision

Samples representing 6 different analyte concentrations, prepared with or without calibrator spike, were measured in duplicates per run across a minimum of 6 runs to assess intra- and inter-assay precision.

Sample Type	Conc. fg/mL	Mean Intra-assay CV (range)	Inter-assay CV
EDTA Plasma	139	8.3% (2.8 – 20.4%)	8.3%
	162	6.8% (0.7 – 13.4%)	12.7%
	233	6.2% (1.3 – 14.3%)	9.0%
	254	6.3% (1.3 – 14.7%)	8.2%
	380	3.7% (1.6 – 8.7%)	10.6%
	396	8.5% (1.1 – 19.1%)	10.5%

Dilution Linearity

EDTA plasma samples from apparently healthy donors (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 (3 donors)	2x	106% (104 – 109%)	89.2%
	4x	96.3% (94.8 – 97.7%)	
	8x	87.5% (85.9 – 89.7%)	
	16x	80.8% (77.8 – 83.6%)	
	32x	75.5% (71.1 – 79.9%)	

Spike Recovery

To assess recovery in native matrices, EDTA plasma samples from apparently healthy donors (n = 3) were spiked with calibrator at low, medium, and high concentrations (respective to endogenous levels and assay dynamic range). All samples were diluted at the MRD, and 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 (3 donors)	Low	128% (122 -133%)	128%
	Med	127% (121 – 130%)	
	High	131% (122 – 137%)	

Specificity & Interference

Non-phosphorylated full-length Tau was 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.

Potential interference was also 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.

Highest Concentration tested	
Hb	400 mg/dL
TG	500 mg/dL
CB	2.5 mg/dL
UB	2.5 mg/dL

Notes:

- All data shown here were generated in 384-well runs with qPCR amplification on QuantStudio instruments.
- LLoD and LLoQ are post-MRD values.
- PET-confirmed EDTA plasma samples were obtained through the Global Alzheimer's Platform (GAP).
- Endogenous levels for CSF were tested for reference only. Full verification of assay performance is recommended before using CSF matrix in studies.