Quanterix
Discovery Fueled by Ultra-Sensitivity

Simoa® p-Tau 231 Advantage PLUS Kit

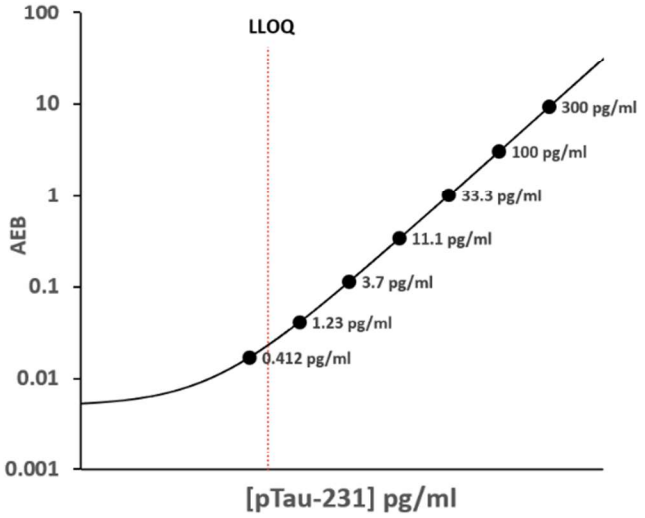
HD-X Data Sheet

Item # 104512

Description

Threonine 231 is one of the phosphorylation sites of human tau protein. Tau is a microtubule-stabilizing protein primarily localized in neurons of the central nervous system but is also expressed at low levels in astrocytes and oligodendrocytes. Tau consists of six isoforms in the human brain with molecular weights of 48,000 to 67,000 Daltons, depending on the isoform.[1] Tau elevation is observed in the cerebrospinal fluid (CSF) of patients with neurodegenerative disease[2,3] and severe head injuries[4] suggesting its extracellular release during neuronal damage and its potential role as a specific biomarker for brain injury. In Alzheimer's disease (AD) and related neurodegenerative diseases, including chronic traumatic encephalopathy (CTE), tau is abnormally phosphorylated and aggregated into bundles of filaments.[5] Phosphorylated tau is believed to be a more relevant biomarker for Alzheimer's disease. Tau phosphorylated at threonine 231 has been shown to differentiate Alzheimer's disease from healthy controls.[6]

Calibration Curve: The Representative calibrator concentrations and Lower Limit of Quantification (LLOQ) depicted. For p-Tau 231 Advantage PLUS, the reconstitution volume for calibrator concentrates may vary between kit lots, while keeping the target calibrator concentrations each level as consistent as possible: The minimum allowable concentration for Cal H is 300 pg/mL and the maximum allowable concentration for Cal B is 0.617 pg/mL.



MRD (Minimum Required Dilution)	
Diluted Sample Volume	100 µL per measurement
Human EDTA Plasma Dilution	1:2
Human CSF Dilution	1:4
Tests per kit	96

See Kit Instruction for details.

Lower Limit of Quantification (LLOQ): Triplicate measurements of serially diluted calibrator were read back on the calibration curve over 12 runs each for 2 reagent lots across 2 instruments (3 runs per lot, per instrument). The analytical LLOQ was set at the lowest concentration that read back within 80 – 120% of the expected value with a CV < 20%. The functional LLOQ (fLLOQ) values for each matrix represent the analytical LLOQ multiplied by the dilution factor used for the respective matrix (Table).

Limit of Detection (LOD): Calculated as 2.5 standard deviations from the mean of background signal read back on each calibration curve over 12 runs for 2 reagent lots across 2 instruments (3 runs per lot, per instrument).

Assay Range: The upper end of the dynamic range, or functional Upper Limit of Quantification (ULOQ) is equal to the minimum top calibrator concentration multiplied by MRD.

Analytical LLOQ	**0.617 pg/mL** pooled CV 20% mean recovery 115%
2X Functional LLOQ (plasma)	**1.23 pg/mL**
2X Functional ULOQ (plasma)	**600 pg/mL**
4X Functional LLOQ (CSF)	**2.47 pg/mL**
4X Functional ULOQ (CSF)	**1200 pg/mL**
LOD	**0.232 pg/mL** range 0.091–0.837 pg/mL

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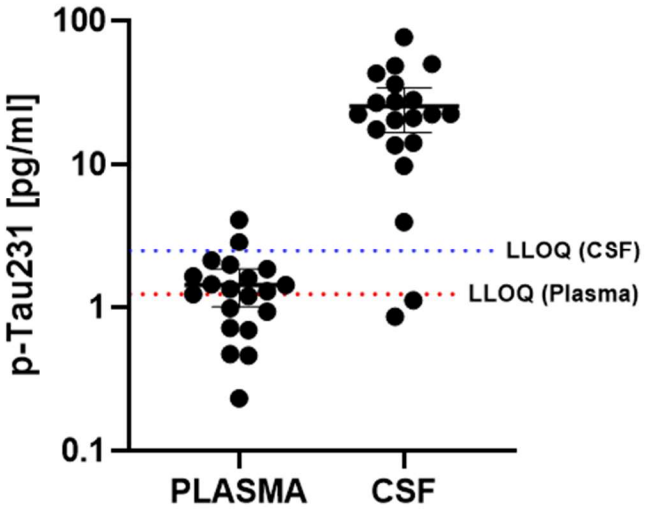
Endogenous Sample Reading: Concentrations (pg/mL) were determined for unmatched EDTA plasma (n=20) from normal human donors and normal human CSF (n=20) using the p-Tau 231 Advantage PLUS kit on HD-X. Bars depict median with interquartile range. The red and blue lines represent functional LLOQ.



Sample Type	Mean p-Tau 231 pg/mL	Median p-Tau 231 pg/mL *	% Above LOD	% Above LLOQ
Human EDTA Plasma	2.02	1.83	78%	40%
Human CSF	31.7	26.5	98%	90%

Precision: Measurements of 2 human EDTA plasma-based panels, and 2 calibrator-based controls were measured for precision. Triplicate measurements were made for 6 runs each for 2 reagent lot across 2 instruments (12 runs total, 36 measurements). All samples were diluted at the appropriate MRD for the sample matrix.

Sample	Mean (pg/mL)	Within run CV	Between run CV	Between Instr CV	Between Lot CV
Control 1	3.34	8.6%	10.5%	4.4%	1.1%
Control 2	260	2.8%	6.6%	2.0%	2.0%
Panel 1	45.9	4.0%	9.1%	7.7%	1.0%
Panel 2	151	3.6%	10.1%	6.4%	0.1%

Spike and Recovery: Two normal human samples for EDTA plasma were spiked at high (800 pg/mL) and low (160 pg/mL) concentrations within the range of the assay and analyzed on HD-X. Percent recovery is defined as the difference between the measured concentration of p-Tau 231 in the spiked sample and the measured concentration in unspiked sample relative to the concentration of p-Tau 231 in spiked calibrator diluent.

Dilution Linearity: Two normal human plasma samples spiked with recombinant analyte and 2 unspiked normal human CSF samples were diluted 2x serially with sample diluent through 7 levels. Each human plasma dilution series was run on the HD-X with two different lots of p-Tau 231 Advantage PLUS assay kits with the MRD (2X) dilution applied. The total dilution of each human EDTA plasma sample ranged from 2X to 64X. Each human CSF dilution series was run on the HD-X with the MRD (4X) dilution applied. Total dilution of each CSF sample ranged from 4X to 32X. The average percent recovery across the entire dilution series is displayed for each sample type.

Spike and Recovery (Human EDTA Plasma)	**Mean 78.5% Range 64.1–99.8%**
Dilution Linearity (Spiked Human EDTA Plasma, 2X – 64X)	**Mean 114% Range 91.3–147%**
Dilution Linearity (Endogenous Human CSF, 4X – 32X)	**Mean 114% Range 104–119%**

References

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4. Franz G, Beer R, Kampfl A, Engelhardt K, Schmutzhard E, Ulmer H, et al. Amyloid beta 1-42 and tau in cerebrospinal fluid after severe traumatic brain injury. Neurology 2003; 60(9):1457–61.
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6. Hampel H, Blennow K, Shaw LM, et al. Total and Phosphorylated Tau Protein as Biological Markers of Alzheimer's Disease. Exp Gerontol. 2010. 45(1): 30.

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