Simoa® p-Tau 181 Advantage PLUS Kit
HD-X Data Sheet

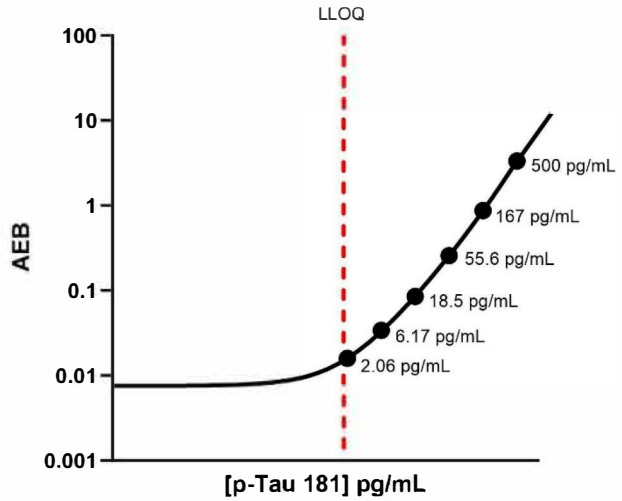
Item 104618

Description

This datasheet summarizes data from analytical validation performed at Quanterix to characterize performance of the p-Tau 181 Advantage PLUS kit on the HD-X platform.

Threonine 181 is one of the phosphorylation sites of the human tau protein (p-Tau 181). Tau is a microtubule-stabilizing protein primarily localized in neurons of the central nervous system but 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 in isoform. The Simoa p-Tau 181 assay targets the proline rich region of the Tau protein which is highly conserved amongst these isoforms. Tau elevation is observed in the cerebrospinal fluid (CSF) of patients with neurodegenerative disease and severe head injuries, suggesting its extracellular release during neuronal damage and a role as a biomarker with specificity for brain injury. In Alzheimer's disease (AD) and related neurodegenerative diseases, including chronic traumatic encephalopathy, tau is abnormally phosphorylated and aggregated into bundles of filaments. p-Tau 181 has been found to be more strongly associated with markers of AD than total tau.

Calibration Curve: Representative calibrator concentrations and Lower Limit of Quantification (LLOQ) depicted. For p-Tau 181 Advantage PLUS, the reconstitution volume for calibrator concentrates may vary between kit lots, while keeping the target calibrator concentrations of each level as consistent as possible: The minimum allowable concentration for Cal G is 500 pg/mL and the maximum allowable concentration for Cal B is 2.06 pg/mL.



Minimum Required Dilution (MRD)

Diluted Sample Volume	100 µL per measurement
Human Serum and EDTA Plasma Dilution	1:4
Human CSF Dilution	1:10
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 lot 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 below are for serum and plasma and represent the analytical LLOQ multiplied by the dilution factor used for the samples. The fLLOQ for CSF is 10x the analytical LLOQ.

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 each for 2 reagent lot 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.

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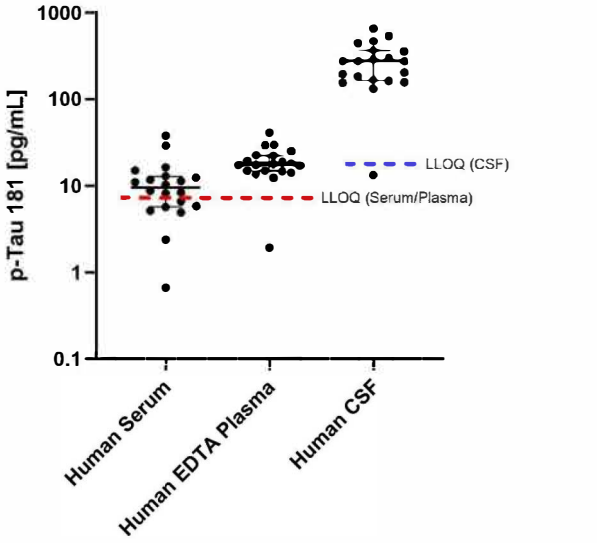
Analytical LLOQ	**1.82 pg/mL** Pooled CV 16.0% Mean Recovery 100% Range: 54 – 150%
Functional LLOQ (serum and plasma)	**7.27 pg/mL**
Functional ULOQ (serum and plasma)	**2000 pg/mL**
Functional LLOQ (CSF)	**18.2 pg/mL**
Functional ULOQ (CSF)	**5000 pg/mL**
LOD	**0.724 pg/mL** Range 0.230–1.67 pg/mL

Endogenous Sample Reading: Concentrations (pg/mL) were determined for matched human EDTA plasma (n=20) and human serum (n=20), and unmatched normal human CSF (n=20) using the p-Tau 181 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 181 pg/mL	Median p-Tau 181 pg/mL	% Above LOD	% Above LLOQ
Human Serum**	14.9	9.54	95%	65%
Human EDTA Plasma**	20.2	17.8	100%	95%
Human CSF	295	276	100%	95%

***Values below LLOQ are not included in the mean.**

****Comparable % Above LOD and % Above LLOQ were determined between the Advantage V2.1 assay and Advantage PLUS assay. For further comparability data between the two assays, see p-Tau 181 Advantage PLUS Technical Note (TECH-0168).**

Precision: Measurements of 1 serum-based panels, 2 plasma-based panels, 1 CSF-based panel and 2 calibrator-based controls. 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 Inst CV	Between Lot CV
Control 1	65.8	6.9%	11.1%	1.1%	4.0%
Control 2	1477	2.5%	5.5%	0.02%	2.5%
Panel 1	17.9	14.3%	13.7%	6.0%	2.4%
Panel 2	16.3	16.3%	9.3%	4.6%	0.7%
Panel 3	743	2.8%	4.6%	2.0%	1.6%
Panel 4	455	3.9%	8.1%	2.9%	0.2%

Spike and Recovery: Spike recovery was measured with human serum and EDTA plasma samples in the feasibility phase of assay development. Results indicate that matrix effects are observed with this assay, as a limited dilution was chosen to maximize the detectability/quantifiability of the analyte in serum and EDTA plasma samples from healthy donors.

Dilution Linearity: Dilution linearity was measured with human serum, EDTA plasma, and CSF samples in the feasibility phase of assay development. Likely due to matrix effects, p-Tau 181 readings do not exhibit linearity upon dilution of the matrix with sample diluent. For valid comparison between results, it is recommended to run all samples at a consistent dilution.

Admixture Linearity: Samples with high concentrations of analyte were created by spiking with calibrator concentrate. One human EDTA plasma and one serum sample were mixed with low-analyte samples of the same matrix at 12 different ratios. The dilution series was run on the HD-X with the MRD

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(4X) dilution applied. Data was generated with two different lots of kits.

Admix Linearity Plasma **Low matrix 10.1 pg/mL** **High matrix 1017 pg/mL**	**Mean 93%** Range 87 – 100% Slope = 1.0259, R^2 = 0.9957
Admix Linearity Plasma **Low matrix 11.1 pg/mL** **High matrix 931 pg/mL**	**Mean 93%** Range 86 – 103% Slope = 1.0124, R^2 = 0.9964
Admix Linearity Serum **Low matrix 18.3 pg/mL** **High matrix 919 pg/mL**	**Mean 94%** Range 88 – 107% Slope = 1.0726, R^2 = 0.9913
Admix Linearity Serum **Low matrix 14.4 pg/mL** **High matrix 761 pg/mL**	**Mean 104%** Range 98 – 110% Slope = 1.0027, R^2 = 0.9981

Calibrator and Sample Stability: Measurements were obtained with 4 human EDTA plasma samples and 7 calibrators. Triplicate measurements were made for 3 stability time points (3-hr and 7-hr stability at room temperature and 24-hr stability at 4°C) on the same instrument using one lot of reagents.

Drift: Measurements of one endogenous human serum sample, one spiked human serum sample, and 2 calibrator-based controls were obtained on the HD-X. Controls and samples were run across three plates with a total of 54 replicates per sample.

Calibrator Stability	**Mean 103%** Range 93.6% - 116%
Sample Stability	**Mean 91.4%** Range 58.9 – 119%
Drift (Three-plate Variance)	**Plate 1:** 9.5% **Plate 1+2:** 8.3% **Plate 1+2+3:** 5.9%
Drift (Three-plate Precision)	**Mean 7.4%** Range 5.1 – 12.0%

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