

ALZpath Simoa® pTau-217 v2 Assay

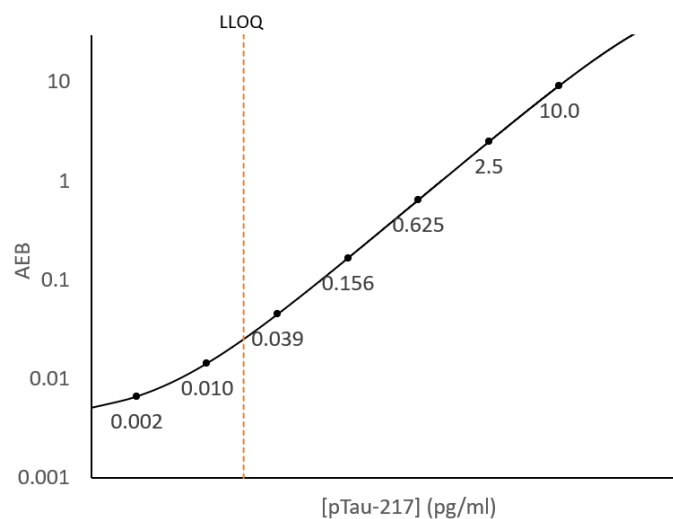
Overview

This technical note describes representative assay performance for the ALZpath Simoa pTau-217 v2 Assay kit in human EDTA plasma samples using the HD-X Analyzer. This assay is for research use only, not for diagnostic use. Assay performance specifications and claims for the assay are representative of the results generated with reagent lots produced by the Quanterix Accelerator Laboratory. The design and configuration of the assay may be subject to change.

Description

Threonine 217 is one of the phosphorylation sites of human tau protein (pTau-217). 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. In Alzheimer's disease (AD) and related neurodegenerative diseases, tau is abnormally phosphorylated and aggregated into bundles of filaments. The ALZpath Simoa pTau-217 v2 Assay is a digital immunoassay for the quantitative determination of phosphorylated tau protein including pTau-217 in human EDTA plasma samples.

Calibration Curve: Calibrator concentrations and Lower Limit of Quantification depicted below.



Minimum Required Dilution (MRD)

Sample Matrix	EDTA Plasma
Diluted Sample Volume	100 µL per measurement
Minimum Required Dilution (MRD)	1:3

Limit of Detection (LOD): 20 replicates of Calibrator A (blank calibrator diluent) were measured with two reagent lots to determine the Limit of Blank (LOB). To determine the LOD, one plate was run with each of two reagent lots with the following samples: 3 samples of Calibrator A (blank), run in singlicate; 3 plasma samples near LOD, run in duplicate.

Lower Limit of Quantification (LLOQ): 5 plasma samples were measured in duplicate in 5 runs over 5 days with each of two reagent kit lots. The pooled values of both kit lots were used to determine the LLOQ, which corresponds to the smallest concentration with a CV less than 20%.

Assay Range: The upper end of the dynamic range is equal to the top calibrator concentration multiplied by MRD.

LOD	Range 0.0052-0.0074 pg/mL*
Analytical LLOQ	0.0200 pg/mL
Functional LLOQ	0.0600 pg/mL
Dynamic Range	0.0070 – 30.0 pg/mL (with 3x dilution)

*Concentration is not dilution corrected

Precision: 3 plasma samples (high, medium, low) were measured in duplicate over at least 20 days on three HD-X Analyzer instruments with two reagent lots, depicted below. Inter-precision of samples was calculated for interpolated concentrations over all 40 runs. All samples were diluted at MRD (3-fold). Two levels of Endogenous QC (EQC) samples measured in duplicate over 5 days with two reagent lots.

Sample	Avg Conc (pg/mL)*	CV
Plasma 1 (High)	1.58	14.46%
Plasma 2 (Medium)	0.419	15.95%
Plasma 3 (Low)	0.034	20.33%
EQC 1	0.211	10.73%
EQC 2	0.431	15.00%

*Average concentration is not dilution corrected

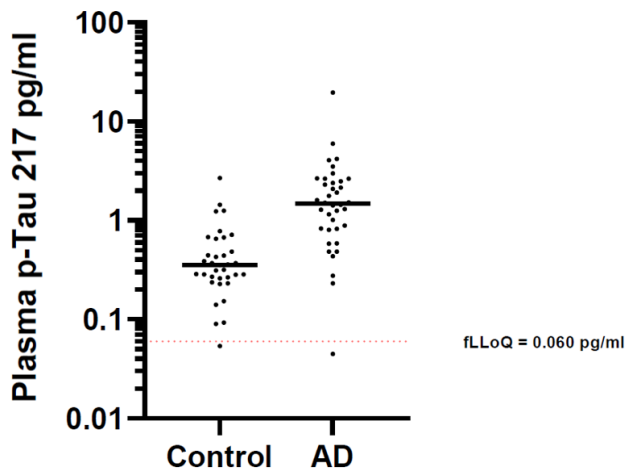
Repeatability: Intra-assay precision was analyzed by performing two runs with each of three plasma samples (high, medium, low) diluted 20 times independently and each dilution run in duplicate with two reagent lots.

Lot	Sample	CV
1	Plasma 1	5.84%
1	Plasma 2	6.84%
1	Plasma 3	2.80%
2	Plasma 1	4.85%
2	Plasma 2	5.00%
2	Plasma 3	3.73%

Sample Stability: Sample stability was evaluated by running four EDTA plasma samples over multiple time and temperature conditions: room temperature for 4 and 8 hours; 2-8°C for 24 and 48 hours. A freeze/thaw study was also conducted by thawing sample aliquots for ≤ 1 hour at room temperature and then freezing for ≥ 12 hours, for between 1-3 cycles. All samples were compared to a freshly-thawed baseline on the same run. Samples were run in duplicate per condition.

Sample	Average	Range
FT1	98.80%	94.62 – 105.83%
FT2	100.80%	99.54 – 103.04%
FT3	102.70%	99.48 – 105.09%
4°C for 24hr	100.75%	98.18 – 105.00%
4°C for 48 hr	99.77%	97.06 – 103.42%
RT for 4 hr	103.31%	97.76 – 107.95%
RT for 8 hr	102.24%	96.52 – 108.08%

Endogenous Sample Reading: Plasma p-Tau levels measured with the ALZpath Simoa p-Tau 217 v2 Assay in samples from the Emory Health Brain Study and Goizueta Alzheimer Disease Research Center (courtesy of Drs. Lah, Levey & Roberts). Samples from healthy controls (n=40) and Alzheimer’s Disease cases (n=38) are shown along with the functional LLoQ of the assay.



Summary

The ALZpath Simoa p-Tau 217 v2 Assay designed for processing on the HD-X Analyzer enables researchers to measure levels of phosphorylated tau protein in EDTA plasma samples with high sensitivity, high precision, and repeatability. The differentiation of healthy controls from AD patient samples offers the opportunity to utilize the assay for routine blood-based biomarker analysis in clinical and translational research applications.