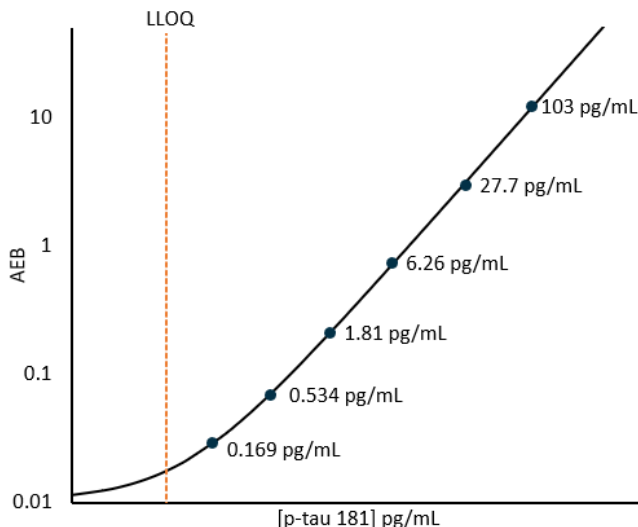


Description

Threonine 181 is one of the phosphorylation sites of human tau protein (pTau-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 on isoform. The Simoa pTau-181 Advantage V2 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. pTau-181 has been found to be more strongly associated with markers of AD than total tau.

Calibration Curve: Calibrator concentrations and Lower Limit of Quantification depicted. (calibrator levels may change for different manufacturing lot)



Minimum Required Dilution (MRD)

| | |
|----------------------------------|------------------------|
| Diluted Sample Volume | 100 uL per measurement |
| Serum and Plasma Dilution | 1:4 |
| CSF Dilution | 1:10 |
| Tests per kit | 96 |

See Kit Instruction for details.

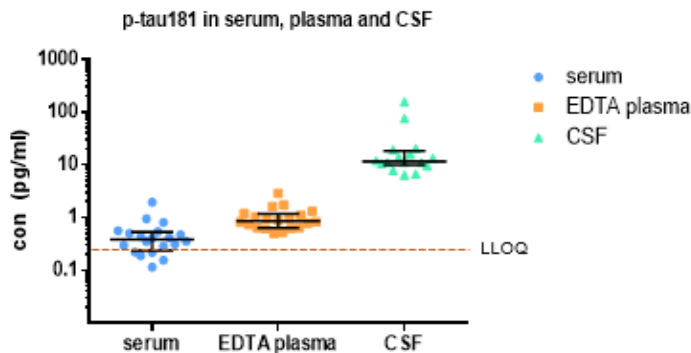
Lower Limit of Quantification (LLOQ): Triplicate measurements of serially diluted calibrator were read back on the calibration curve over 6 runs each for 1 reagent lot across 3 instruments (6 runs total). The functional LLOQ (fLLOQ) values below are for serum and plasma. The fLLOQ for CSF is 2.5x the fLLOQ for serum and plasma.

Limit of Detection (LOD): Calculated as 2.5 standard deviations from the mean of background signal read back on each calibration curve over 6 runs each for 1 reagent lot across 2 instruments (6 runs total).

Assay Range: The upper end of the dynamic range is equal to the top calibrator concentration multiplied by MRD. The ranges below are for serum and plasma. The Upper Limit of Quantification (ULOQ) for CSF is 2.5x the ULOQ for serum and plasma.

| | |
|---------------------------------------|---|
| Analytical LLOQ | 0.0668 pg/mL pooled CV 14.9% mean recovery 96.2% |
| Functional LLOQ (Serum/Plasma) | 0.267 pg/mL |
| LOD | 0.0243 pg/mL range 0.0193-0.0343pg/mL |
| Dynamic Range (Serum/Plasma) | 0 – 412 pg/mL |

Endogenous Sample Reading: Healthy donor matched EDTA plasma (n=20) and serum (n=20) and normal CSF (n=16) samples were measured. Bars depict median with interquartile range. Orange line represents functional LLOQ.



| Sample Type | Mean pTau-181 (pg/mL) | Median pTau-181 (pg/mL) | % Above LOD | % Above LLOQ |
|-------------|-----------------------|-------------------------|-------------|--------------|
| Serum | 0.479 | 0.384 | 100% | 80% |
| EDTA Plasma | 1.02 | 0.856 | 100% | 100% |
| CSF | 25.2 | 11.6 | 100% | 100% |

Precision: Measurements of 1 spiked serum panel, 1 endogenous plasma panel, 1 endogenous CSF panel, and 2 calibrator-based controls. Triplicate measurements were made for 6 runs each for 1 reagent lot across 2 instruments (6 runs total, 18 measurements).

| Sample | Mean (pg/mL) | Within run CV | Between run CV | Between inst CV |
|-----------|--------------|---------------|----------------|-----------------|
| Control 1 | 2.95 | 3.5% | 6.0% | 4.8% |
| Control 2 | 49.4 | 3.2% | 6.5% | 7.5% |
| Panel 1 | 0.506 | 14.3% | 9.0% | 1.2% |
| Panel 2 | 1.28 | 7.1% | 8.2% | 0.6% |
| Panel 3 | 2.31 | 6.4% | 13.9% | 4.2% |

Spike and Recovery: 2 serum and 2 EDTA plasma samples were spiked at high and low concentrations within the range of the assay and analyzed on HD-1/HD-X.

Dilution Linearity: 2 endogenous and 2 spiked EDTA plasma samples, and 1 endogenous and 2 spiked serum samples were diluted 2x serially from MRD (4x) to 128x with Sample Diluent. 2 endogenous CSF samples were diluted 2x serially from MRD (10x) to 160x with Sample Diluent.

| | |
|--|---|
| Spike and Recovery (Serum/Plasma) | Mean 98.8% range 85.9%–112% |
| Dilution Linearity (Serum/Plasma) | Mean 116% range 72.4%–144.2% |
| Dilution Linearity (CSF) | Mean 108.1 Range 99.7%–117.4% |

The Simoa pTau-181 Advantage V2 assay kit is formulated for use on the SR-X, HD-1, or HD-X platform.