# Simoa® SNAP-25 Advantage V2 Kit
## HD-X Data Sheet

Item 104955

### Description

This data sheet summarizes data from the analytical validation performed at Quanterix to characterize performance of the SNAP-25 Advantage V2 kit on the HD-X platform.

### SNAP-25

The Simoa® SNAP-25 Advantage V2 assay targets the soluble N-terminal fragment of SNAP-25 (aa 2-47)-which has been shown to distinguish between the Alzheimer's disease (AD) patients and the nondemented controls. SNAP-25 (Synaptosomal-associated protein, 25kDa) is one of the major proteins involved in the formation of the SNARE (Soluble N-ethylmaleimide sensitive factor attachment protein receptors) protein complex. The formation of the SNARE complex is a crucial step in the process of exocytosis, specifically the release of neurotransmitters at the synapse. SNAP-25 also plays a significant role in processes like neurite extension, neuron repair, synaptogenesis, and the formation of long-term memory through its involvement in Long-Term Potentiation (LTP) - essentially contributing to the mechanisms underlying learning and memory consolidation. Cognitive decline in Alzheimer's disease can be predicted by synaptic dysfunction and degeneration. Synaptic damage can be detected at the earliest stages of AD. Mild Cognitive Impairment (MCI) patients exhibit a significant loss of presynaptic proteins like synaptophysin SNAP-25 and Post-synaptic density proteins like PSD-95 and Shank. Synaptic loss is closely related to severity of clinical disease, and therefore SNAP-25 may be a good biomarker for early diagnosis and as a disease progression predictor. SNAP-25 levels correlate with levels of T-tau and P-tau in control groups and patients with dementia due to AD. Therefore, SNAP-25 may be an important alternate marker in future clinical studies with tau-based-modifying drugs

**Calibration Curve:** The representative calibrator concentrations and Lower Limit of Quantification (LLOQ) are depicted in Figure 1. The reconstitution volume and the assigned concentrations of calibrator levels may vary between different kit lots.

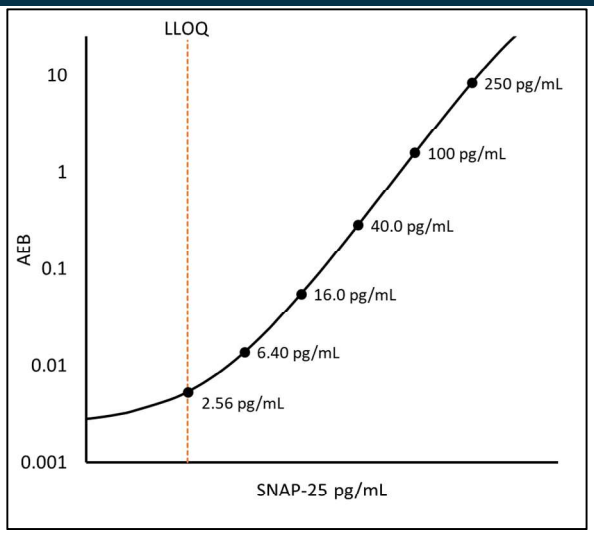



Figure 1. Example calibrator curve.

### Minimum Required Dilution (MRD)

| Diluted Sample Volume | 100 µL per measurement |
|---|---|
| CSF Dilution | 1:4 |
| Tests per Kit | 96 |

See Kit Instruction for details.

**Limit of Quantification (LOQ)**: The analytical LLOQ is determined as the lowest concentration of the analyte in Sample Diluent with a recovery between 80 – 120% and a CV < ±20%. The functional LLOQ (fLLOQ) is the analytical LLOQ multiplied by MRD. The analytical ULOQ (ULOQ) is the concentration of the highest calibrator. The functional ULOQ (fULOQ) is the analytical ULOQ multiplied by the MRD.

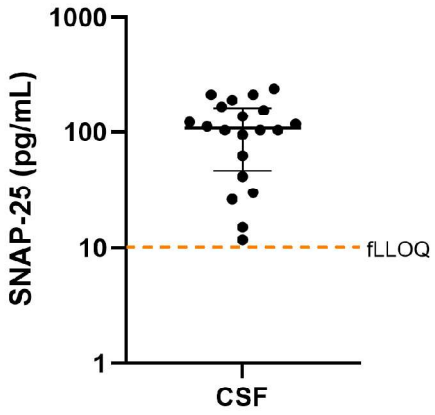
**Limit of Detection (LOD):** The limit of detection is calculated as 2.5 standard deviations from the mean of background signal read back on each calibration curve.

Quanterix Corporation
900 Middlesex Turnpike, Billerica, MA 01821
techsupport@quanterix.com

**Assay Range:** The upper end of the dynamic range is equal to the top calibrator concentration multiplied by MRD. The representative ranges below are for CSF.

| SNAP-25 | |
|---|---|
| Analytical LLOQ | 2.56 pg/mL<br>Pooled CV: 16.2%<br>Recovery: 101% |
| Functional LLOQ | CSF (4x): 10.2 pg/mL |
| LOD | 1.47 pg/mL<br>Range: 0.999 – 2.02 pg/mL |
| Dynamic Range | CSF (4x): 0 - 1000 pg/mL |

**Endogenous Sample Reading:** Healthy donor CSF (n=20) concentrations (pg/mL) were measured using the SNAP-25 Advantage V2 kit on HD-X. Bars depict median with interquartile range. The orange line is a representative value functional LLOQ, which may vary from lot to lot.




| SNAP-25 | | | | |
|---|---|---|---|---|
| Sample Type | Mean (pg/mL) | Median (pg/mL) | % Above LOD | % Above fLLOQ |
| CSF | 113 | 108 | 100% | 100% |

**Precision:** Measurements of 2 calibrator-based controls and 2 CSF samples. Triplicate measurements were made for 6 runs each for 1 reagent lot across 2 instruments (6 runs total, 18 measurements). All samples were diluted at the appropriate MRD for the sample matrix.

| SNAP-25 | | | | |
|---|---|---|---|---|
| Sample | Mean (pg/mL) | Within Run CV | Between Run CV | Between Instrument CV |
| Control 1 | 52.1 | 1.6% | 4.4% | 0.8% |
| Control 2 | 408 | 1.7% | 2.4% | 0.4% |
| CSF 1 | 72.9 | 3.3% | 4.5% | 4.6% |
| CSF 2 | 561 | 1.8% | 3.0% | 2.2% |

**Spike and Recovery:** 4 CSF samples were spiked at high and low concentrations of SNAP-25 within the range of the assay and analyzed on HD-X. Percent recovery is defined as the difference between the measured concentration of the analytes in the spiked sample and the measured concentration in unspiked sample relative to the concentration of the analytes in spiked sample diluent.

**Dilution Linearity:** 4 CSF samples were serially diluted 2x with sample diluent. The assay was determined to be linear from 4x to 64x dilution.

| SNAP-25 | |
|---|---|
| Spike and Recovery<br>(CSF, 100 and 500 pg/ml) | Mean: 88.6%<br>Range: 73.0 – 104% |
| Dilution Linearity<br>(CSF, 4X-64X) | Mean: 103%<br>Range: 85.9 – 123% |

The Simoa® SNAP-25 Advantage V2 assay kit is formulated for use on the HD-X platform. Verification and validation results for the fully automated HD-X instrument are summarized in this report.

Quanterix Corporation
900 Middlesex Turnpike, Billerica, MA 01821
techsupport@quanterix.com