## Description

This datasheet summarizes data from analytical validation performed at Quanterix to characterize performance of the PSD-95 Advantage PLUS kit on the HD-X platform.

The postsynaptic density (PSD) is a massive protein complex, critical for synaptic strength and plasticity in neurons. PSD-95 (postsynaptic density protein 95) is a membrane-associated guanylate kinase (MAGUK) scaffolding protein encoded by the DLG4 gene and is associated with excitatory synapses. Recent studies present PSD-95 as an emerging biomarker in Alzheimer's disease (AD), levels of which correlate with neuronal loss, cognitive decline, and other measures of synaptic plasticity. Abnormalities in PSD-95 expression result in disruptions of the ion channels directly causing channelopathy-induced disorder and symptoms of Alzheimer's disease. Mutations in DLG4 gene results in the development of other neurological disorders, such as Shine syndrome.

Calibration Curve: Representative calibrator concentrations and Lower Limit of Quantification (LLOQ) depicted. For PSD-95 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.
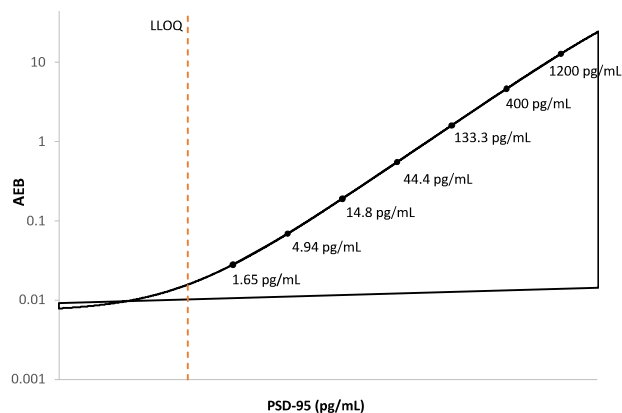
Minimum Required Dilution (MRD)

| Diluted Sample Volume	100 µL per measurement
Serum, EDTA Plasma, and 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 6 runs each for 2 reagent lots across 3 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, EDTA plasma, and CSF and represent the analytical LLOQ multiplied by the dilution factor used for the samples.

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 2 reagent lots across 3 instruments (3 runs per lot, per instrument).

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 serum, EDTA plasma and CSF.

Analytical LLOQ	**0.823 pg/mL** pooled CV 15% mean recovery 98%
Functional LLOQ (Serum, EDTA Plasma, and CSF)	**3.292 pg/mL**
Functional ULOQ (Serum, EDTA Plasma, and CSF)	**4800 pg/mL**
LOD	**0.173 pg/mL** Range: 0.041-0.346 pg/mL
Dynamic Range (Serum, EDTA Plasma, and CSF)	**0 –4800 pg/mL**

Endogenous Sample Reading: Healthy donor matched EDTA plasma (n=20), and serum (n=20), and unmatched CSF (n=20) samples were measured. Bars depict median with interquartile range. Orange line represents functional LLOQ.
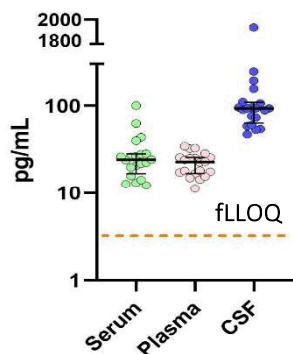
Sample Type	Mean pg/mL	Median pg/mL	% Above LOD	% Above LLOQ
Serum	29.26	24.18	100%	100%
EDTA Plasma	22.32	22.95	100%	100%
CSF	190.94	93.27	100%	100%

Precision: Measurements of 3 serum-based panels, 3 EDTA plasma-based panels and 2 calibrator-based controls. Triplicate measurements were made for 6 runs each for 2 reagent lots across 3 instruments (12 runs total, 96 measurements). All samples were diluted at the appropriate MRD for the sample matrix.

Sample	Mean (pg/mL)	Within run CV	Between run CV	Between lot CV	Between inst CV
Control 1	29.59	5%	8%	4%	6%
Control 2	679.38	6%	18%	3%	2%
Panel 1	28.28	4%	9%	7%	7%
Panel 2	67.61	5%	8%	2%	5%
Panel 3	1283.66	5%	10%	1%	7%
Panel 4	33.37	3%	7%	1%	6%
Panel 5	78.30	2%	8%	0%	8%
Panel 6	1218.97	3%	6%	2%	8%

Spike and Recovery: 4 serum, 4 EDTA plasma, and 4 CSF samples were spiked at high (1450 pg/mL) and low (290 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 PSD-95 in the spiked sample and the measured concentration in unspiked sample relative to the concentration of PSD-95 in spiked calibrator diluent. Results indicate that Matrix effects are observed with this assay for serum and EDTA plasma samples, as a limited dilution was chosen to maximize the detectability / quantifiability of the analyte in samples from healthy donors.

Dilution Linearity: 2 serum, 2 EDTA plasma and 4 CSF samples were serially diluted with sample diluent through 5 levels of 2X dilutions. Each dilution series was run on the HD-X with the MRD (4x) dilution applied. Total dilution of each sample ranged from 4x to 128x.

Spike and Recovery (Serum)	**Mean 38%** range 25–61%
Spike and Recovery (EDTA Plasma)	**Mean 44%** range 35–60%
Spike and Recovery (CSF)	**Mean 96%** range 87–121%
Dilution Linearity (Serum; 4x - 128x)	**Mean 107%** range 98–124%
Dilution Linearity (EDTA Plasma; 4x - 128x)	**Mean 108%** range 96–125%
Dilution Linearity (CSF; 4x – 128x)	**Mean 99%** range 70–121%

The Simoa PSD-95 Advantage PLUS assay kit is formulated for use on the HD-X platform. Some differences in performance claims between the HD and SR-X platforms may be observed when comparing data sheets for these platforms. This may be due to experiments run at different time-points with different reagent lots and different samples or may be due to minor differences in antibody and analyte behavior in the different assay formats.