Simoa® NPTX2 Advantage PLUS Kit
HD-X Data Sheet

Item 106101

Description: This data sheet summarizes data from the analytical validation performed at Quanterix to characterize performance of the NPTX2 Advantage PLUS kit on the Simoa HD-X Analyzer®.

Matrix Types: Assay performance and a recommended dilution were evaluated in multiple matrices (Table 1). See the Kit Instructions for sample and dead volume requirements.

Table 1. Recommended dilution

Matrix Types	CSF, Serum, EDTA Plasma
Diluted Sample Volume	100 uL per measurement
Human CSF Recommended Dilution	8x
Human Serum Recommended Dilution	32x
Human EDTA Plasma Recommended Dilution	32x

Calibration Curve: The reconstitution volume, assigned concentrations, Limit of Detection (LOD), analytical Upper Limit of Quantification (ULOQ), analytical Lower Limit of Quantification (LLOQ) described here are representative and may vary from kit lot to kit lot (Figure 1). Refer to the Certificate of Analysis (CoA) for lot-specific calibrator concentrations and reconstitution volumes.

Limit of Quantification (LOQ): The analytical LLOQ was determined as the lowest concentration of the analyte in Sample Diluent with a recovery between 80 – 120% and a CV < ±20%. The analytical ULOQ (ULOQ) is the concentration of the highest calibrator. The analytical LLOQ and the analytical ULOQ multiplied by the MRD yields the functional LLOQ (fLLOQ) and the functional ULOQ (fULOQ). The LLOQ was experimentally verified for each kit lot. Minor variations in ULOQ between kit lots may be observed where lot matching was performed (Table 2).

Limit of Detection (LOD): The LOD was calculated as 2.5 standard deviations above the mean of the background (Cal A) (Table 2).

Table 2. LLOQ and LOD

	Analytical	Functional
LLOQ	2.9297 pg/mL	CSF (8x): 23.4376 pg/mL Serum (32x): 93.7504 pg/mL EDTA Plasma (32x): 93.7504 pg/mL
ULOQ	3000 pg/mL	CSF (8x): 24000 pg/mL Serum (32x): 96000 pg/mL EDTA Plasma (32x): 96000 pg/mL
LOD	0.8646 pg/mL	N/A

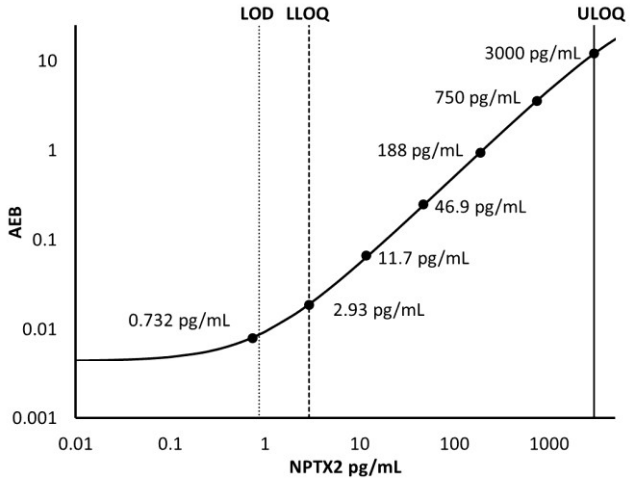

Figure 1. Example calibrator curve

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Reference Ranges: Range was established using normal human CSF (n=19) and matched serum (n=20) and EDTA plasma (n=20). The mean and median analyte concentrations and the percent of samples above the fLLOQ and LOD are reported below (Figure 2 and Table 3).

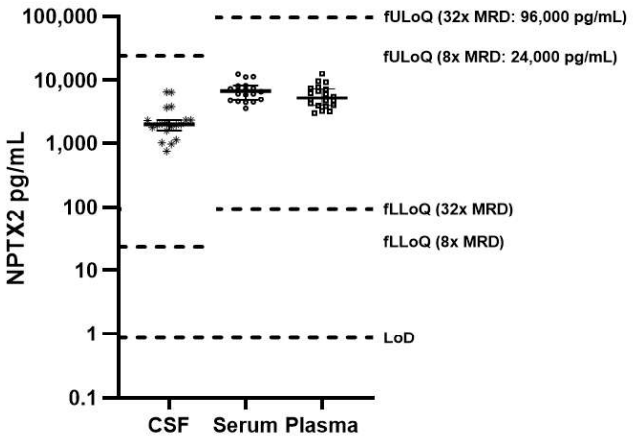

Figure 2. Normal sample readings. Bars depict the median with the interquartile range.

Table 3. Normal sample readings

Sample Type	Mean pg/mL	Median pg/mL	% Above LOD	% Above fLLoQ
CSF	2624.4494	2185.5329	100%	100%
Serum	7455.5185	6620.3448	100%	100%
EDTA Plasma	5945.6154	5359.4930	100%	100%

Precision: Precision was calculated using artificial CSF, commercial pooled serum, commercial pooled plasma, and assay kit controls diluted to the appropriate recommended dilution (Table 4). Triplicate measurements were made across 12 runs. Two reagent lots were each tested 6 times across 2 instruments, providing a mean of 36 individual measurements. Four distinct precision values were calculated from the 36 measurements:

1. **Within Run %CV** describes the variability of the %CV within a run, inclusive of 2 reagent lots and 2 instruments.
2. **Run to Run Conc %CV** describes the variability of the concentration value across all 12 runs, inclusive of 2 reagent lots and 2 instruments.
3. **Instrument %CV** describes instrument-to-instrument variability.
4. **Lot %CV** describes lot-to-lot variability.

Table 4. Precision

Sample	Mean (pg/mL)	Within Run %CV	Run to Run Conc %CV	Instrument %CV	Lot %CV
Control 1	215.4706	4.3%	12.9%	3.5%	2.5%
Control 2	5346.4157	3.7%	10.0%	8.4%	0.3%
CSF 1	333.0763	3.1%	11.3%	4.8%	2.5%
CSF 2	14640.7902	2.6%	11.1%	8.5%	3.6%
Serum 1	5546.7314	3.5%	7.4%	6.4%	0.7%
Serum 2	28475.9765	3.4%	8.6%	4.8%	5.6%
Plasma 1	224.1186	6.1%	13.7%	7.5%	10.6%
Plasma 2	5079.3417	2.7%	16.5%	2.3%	15.6%

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Spike Recovery: Percent recovery was calculated as the difference between the spiked (with antigen) sample and the un-spiked sample, relative to the spiked (with antigen) Sample Diluent (Table 5).

Linearity: Normal human CSF, serum, and EDTA plasma were diluted 2x serially with Sample Diluent. Linearity refers to the assay's ability to produce proportional and accurate results across a defined dilution range. Linearity was assessed by performing serial dilutions of samples diluted with Sample Diluent (Table 5).

Table 5. Spike Recovery and Linearity

Spike Recovery (CSF)	**Mean 90.4%** Range 85.1 – 105.3%
Spike Recovery (Serum)	**Mean 84.9%** Range 72.6 – 89.2%
Spike Recovery (EDTA Plasma)	**Mean 72.4 %** Range 64.2 – 77.5%
Linearity Mean (CSF, Linear Range 4x – 64x)	**Mean 110.2%** Range 87.5 - 164.0%
Linearity Mean (Serum, Linear Range 16x – 64x)	**Mean 98.8%** Range 76.5 – 123.9%
Linearity Mean (EDTA Plasma, Linear Range 16x – 64x)	**Mean 97.7%** Range 75.2 – 116.8%

The Simoa® NPTX2 Advantage PLUS assay kit is formulated for use on the Simoa HD-X Analyzer®. Validation results for the Simoa HD-X Analyzer® are summarized in this data sheet.

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