

**Description**

This data sheet summarizes data from the analytical validation performed at Quanterix to characterize performance of the IL-1β Advantage PLUS kit on the HD-X platform.

**IL-1β**

Interleukin-1 beta (IL-1β), also known as catabolin, is a cytokine of 269 amino acids (molecular weight 31 kDa). This cytokine is produced by activated macrophages as a proprotein, which is proteolytically processed to its active form by caspase-1. IL-1β is an important mediator of the inflammatory response and is involved in a variety of cellular activities, including cell proliferation, differentiation, and apoptosis. IL-1β is the most studied member of the IL-1 family of cytokines due to its role in mediating autoinflammatory diseases. Blood monocytes from patients with autoinflammatory syndromes release more processed IL-1β than cells from healthy subjects and thus likely account for the inflammation in these diseases. Neutralization of IL-1β results in rapid and sustained reduction in disease severity. Although some autoinflammatory diseases are due to gain-of-function mutations for caspase-1 activity, common diseases such as gout, type 2 diabetes, heart failure, recurrent pericarditis, rheumatoid arthritis and smouldering myeloma are also responsive to IL-1β neutralization.

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

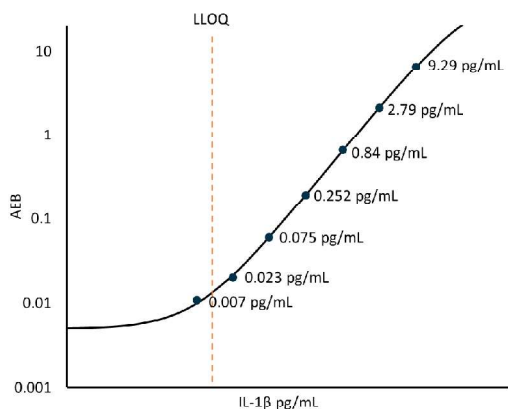


Figure 1. Example calibrator curve

**Minimum Required Dilution (MRD)**

<b>Diluted Sample Volume</b>	100 µL per measurement
<b>Serum/EDTA Plasma Dilution</b>	1:4
<b>Tests per Kit</b>	96

See Kit Instruction for details.

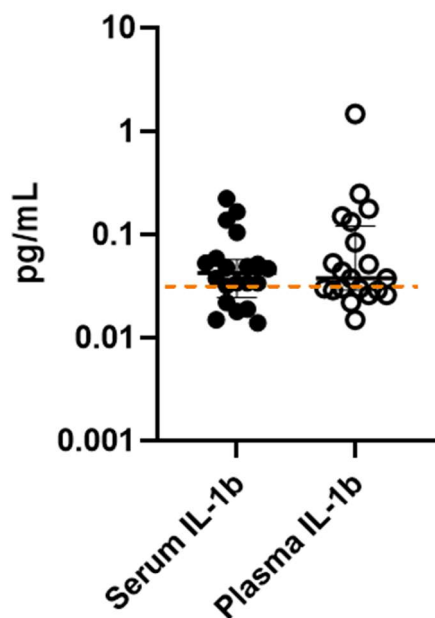
**Lower Limit of Quantification (LLOQ):** The analytical LLOQ was set at the lowest concentration that read back within 80 – 120% of the expected value with a CV ≤ 20%. The functional LLOQ (fLLOQ) values below are for serum and EDTA plasma and represent the analytical LLOQ multiplied by the dilution factor used for the samples.

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

**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 and EDTA plasma.

<b>IL-1β</b>	
<b>Analytical LLOQ</b>	0.008 pg/mL Pooled CV: 17% Mean Recovery: 113%
<b>Functional LLOQ</b>	Serum/EDTA Plasma (4x): 0.031 pg/mL
<b>LOD</b>	0.002 pg/mL Range: 0.001 - 0.003 pg/mL
<b>Dynamic Range</b>	Serum/EDTA Plasma (4x): 0 - 48 pg/mL

**Endogenous Sample Reading:** Healthy donor matched EDTA plasma (n=20), and serum (n=20) concentrations (pg/mL) were measured using the IL-1β Advantage PLUS kit on HD-X. Bars depict median with interquartile range. The orange line is a representative value for functional LLOQ, which may vary from lot to lot.



IL-1β				
Sample	Mean (pg/mL)	Within Run CV	Between Run CV	Between Instr CV
Control 1	0.409	3.6%	7.8%	9.3%
Control 2	24.3	5.5%	15%	8.9%
Plasma 1	0.126	5.9%	15%	6.4%
Plasma 2	7.95	4.1%	8.9%	5.6%
Plasma 3	8.06	3.3%	9.8%	8.5%
Serum 1	0.126	3.6%	14%	8.0%
Serum 2	7.48	4.3%	15%	2.9%
Serum 3	9.24	3.6%	7.6%	7.5%

**Spike and Recovery:** 2 serum and 2 EDTA plasma samples were spiked at high and low concentrations of IL-1β within the range of each 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 calibrator diluent.

**Dilution Linearity:** 2 serum and 2 EDTA plasma samples were spiked with endogenous antigen and then diluted 2x serially with sample diluent. Total dilution of each sample ranged from 4x to 64x.

IL-1β				
Sample Type	Mean (pg/mL)*	Median (pg/mL)*	% Above LOD	% Above fLLOQ
Serum	0.077	0.05	100%	70%
EDTA Plasma	0.227	0.084	100%	55%

\*Values below LLOQ are excluded from the mean and median calculation.

**Precision:** Measurements of 2 calibrator-based controls, 3 commercial pooled serum and 3 commercial pooled plasma. Triplicate measurements were made for 2 runs each for 1 reagent lot across 2 instruments (4 runs total, 12 measurements). All samples were diluted at the appropriate MRD for the sample matrix.

IL-1β †	
Spike and Recovery Serum	Mean: 85% Range: 79 - 97%
Spike and Recovery EDTA Plasma	Mean: 74% Range: 62 - 90%
Dilution Linearity Serum (4X-64X)	Mean: 106% Range: 96 - 130%
Dilution Linearity EDTA Plasma (4X-64X)	Mean: 107% Range: 96 - 120%

† The assay design is conserved between C4PA Advantage PLUS and IL-1β Advantage PLUS. Values are sourced from the C4PA Advantage PLUS Validation report.

The Simoa® IL-1β Advantage PLUS 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.