Quanterix
Discovery Fueled by Ultra-Sensitivity

Simoa® IL-1b 3.0 Advantage Kit
HD-1/HD-X Data Sheet

Item 103464

Description

This datasheet summarizes data from analytical validation performed at Quanterix to characterize performance of the IL-1b 3.0 Advantage kit on the HD–1/HD–X platform.

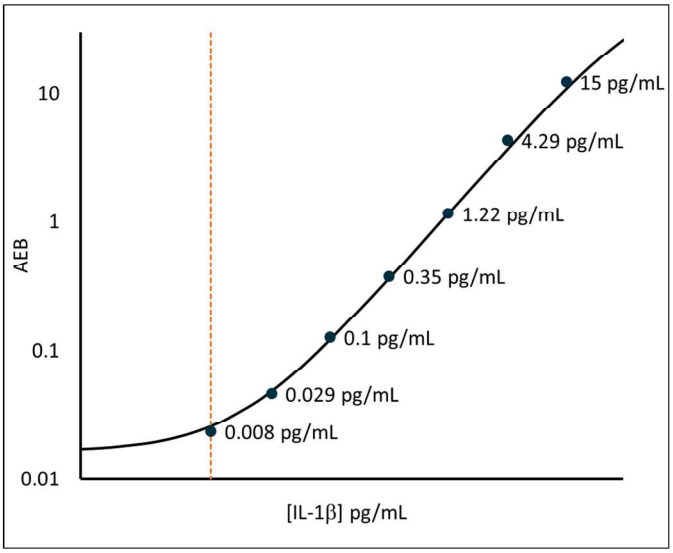
Interleukin-1 beta (IL-1β), also known as catabolin, is a cytokine of 269 amino acids (molecular weight 31 kDa). This cytokine is produced by activated macrophages as a proprotein, which is proteolytically processed to its active form by caspase-1. IL-1β is an important mediator of the inflammatory response 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.

Minimum Required Dilution (MRD)

Diluted Sample Volume	152 µL per measurement
Serum and EDTA Plasma Dilution	1:2
Tests per kit	96

See Kit Instruction for details.

Calibration Curve: Representative 4-parameter calibrator curve and Lower Limit of Quantification (LLOQ) depicted. A calibrator concentrate is provided with the kit. The specific value-assigned concentration of the calibrator concentrate provided on the lot-specific Certificate of Analysis (COA) for the kit, along with the dilution scheme outlined in the Kit Instructions are used to create individual calibrators and controls at the time of kit use.



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 2 instruments (12 runs total). The analytical LLOQ was set at the lowest concentration with average read-back within 80 – 120% of the expected value and average CV ≤ 20%. The functional LLOQ (fLLOQ) values below are for serum and EDTA plasma and represent the analytical LLOQ multiplied by the dilution factor (2x) 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 2 instruments (12 runs total). Reported LOD is the average of the 12 runs.

Assay Range: The upper end of the dynamic range is equal to the top calibrator concentration multiplied by MRD.

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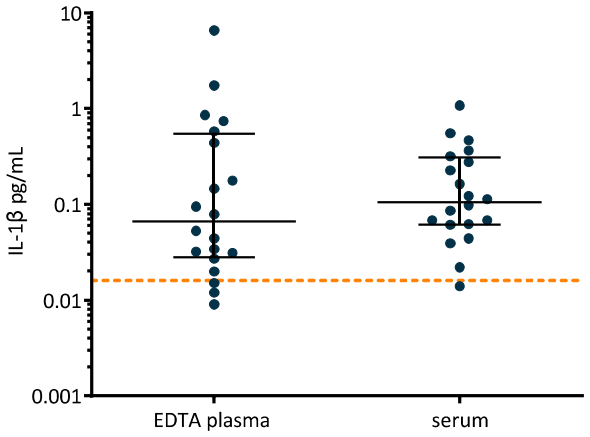
Analytical LLOQ	**0.008 pg/mL** pooled CV 17.3% mean recovery 117%
Functional LLOQ (serum and EDTA plasma)	**0.016 pg/mL**
Functional ULOQ (At the MRD of serum and EDTA plasma)	**30 pg/mL**
LOD	**0.002 pg/mL** range 0.001–0.004 pg/mL
Dynamic Range (serum and EDTA plasma)	**0 – 30 pg/mL**

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



Sample Type	Mean* IL-1β pg/mL	Median IL-1β pg/mL	% Above LOD	% Above LLOQ
Serum	0.223	0.091	100%	95%
EDTA Plasma	0.686	0.057	100%	85%

***Values reading below the LLOQ are not included in the mean**

Precision: Measurements of 4 matrix panels (1 sample each of: unspiked serum, unspiked EDTA plasma, recombinant antigen spiked serum, and recombinant antigen spiked EDTA plasma) and 2 calibrator-based controls. Triplicate measurements were made for 6 runs each for 2 reagent lot across 2 instruments (12 runs total, 36 measurements). All samples were diluted on-board at the appropriate MRD for the sample matrix.

Sample	Mean (pg/mL)	Within run CV	Between run CV	Between inst CV	Between Lot CV
Control 1	0.190	3.5%	7.5%	4.0%	5.6%
Control 2	11.15	4.3%	10.7%	1.9%	11.3%
Panel 1	0.091	5.9%	17.1%	14.5%	15.8%
Panel 2	0.042	11.5%	11.2%	10.2%	1.9%
Panel 3	5.34	5.0%	18.1%	7.1%	21.3%
Panel 4	19.9	7.0%	15.2%	6.3%	16.4%

Spike and Recovery: 2 serum and 2 EDTA plasma samples were spiked at high (1.3 pg/mL) and low (0.26 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 IL-1β in the spiked sample and the measured concentration in unspiked sample relative to the concentration of IL-1β in spiked calibrator diluent. Results indicate that matrix effects are observed with this assay, as a limited dilution was chosen to maximize the detectability / quantifiability of the analyte in samples from healthy donors.

Dilution Linearity: 2 recombinant antigen-spiked EDTA plasma and 2 recombinant antigen-spiked serum samples were serially diluted with sample diluent through 7 levels of 2X dilutions. Each dilution series was run on the HD-X with the MRD (2x) dilution applied. Total dilution of each sample ranged from 2x to 128x.

Admixture Linearity: Samples with high concentrations of analyte were created by spiking with recombinant antigen. 1 spiked EDTA plasma and 1 spiked serum sample were mixed with low-analyte samples of the same matrix at 12 different ratios. The dilution series was run on the HD-X with the MRD (2x) dilution applied.

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Spike and Recovery (Serum)	**Mean 70.1%** range 57.9–87.5%
Spike and Recovery (EDTA Plasma)	**Mean 66.8%** range 56.8–77.8%
Dilution Linearity (EDTA Plasma Donor 1, 2x - 128x)	**Mean 115%** range 90 - 133%
Dilution Linearity (EDTA Plasma Donor 2, 2x - 128x)	**Mean 136%** range 104 - 163%
Dilution Linearity (Serum, 2x - 128x)	**Mean 97%** range 71 - 108%
Dilution Linearity (Serum, 2x - 128x)	**Mean 99%** range 87 - 116%
Admix Linearity EDTA Plasma low matrix 0.072 pg/mL high matrix 22.1 pg/mL	**Mean 93%** range 80 - 102% Slope = 0.94, R2 = 0.99
Admix Linearity Serum low matrix 4.62 pg/mL high matrix 0.03 pg/mL	**Mean 106%** range 91 - 108% Slope = 1.1, R2 = 0.98

Sample Freeze-Thaw Stability: Measurements were obtained with 2 unspiked EDTA plasma and 2 unspiked serum samples. Triplicate measurements were made were made in a single instrument run with sample aliquots that underwent 0, 1, 2, or 3 Freeze-Thaw cycles (in addition to initial freezing). Data is presented as % recovery vs. the aliquot with 0 freeze-thaw cycles.

Sample Freeze-Thaw Stability (EDTA Plasma)	**Mean 105%** range 90 - 120%
Sample Freeze-Thaw Stability (Serum)	**Mean 108%** range 106 - 111%

The Simoa IL-1β assay kit is formulated for use on the SR-X, HD-1, or 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.

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