

Description

This data sheet summarizes data from the analytical validation performed at Quanterix to characterize performance of the IL-22 Advantage PLUS kit on the HD-X platform.

IL-22

IL-22 is a member of the IL-10 superfamily of cytokines. These cytokines are pleiotropic, affecting a wide range of immune functions. IL-22 is produced by Dendritic, T, and Innate Lymphoid cells and can be found in a wide range of tissues. Biological activity of IL-22 is initiated through interactions with IL-22R1 and IL-10R2, as well as IL-22BP1 and is regulated by IL-17A. IL-22 activation plays a role in the initiation and regulation of nonspecific immune response. IL-22 is associated with psoriasis, serum levels of the cytokine correlate with the severity of the disease. Emerging evidence suggests that IL-22 can play a role in other autoimmune disorders such as Inflammatory Bowel Disease, Rheumatoid Arthritis, and Multiple Sclerosis, perhaps due to its role in inflammatory responses, which are regulated by IL-17A. IL-22 has also been implicated as a Reg gene regulator promoting β -cell production in Type 1 diabetes. The Total IL-22 Discovery assay detects free IL-22 and IL-22 bound to IL-22BP.

Minimum Required Dilution (MRD)

Diluted Sample Volume	25 μ L per measurement
Serum and EDTA Plasma Dilution	1:4
Tests per Kit	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 \leq 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): 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.

IL-22	
Analytical LLOQ	0.061 pg/mL Pooled CV: 12% Recovery: 101%
Functional LLOQ	Serum/EDTA Plasma (4x): 0.243 pg/mL
Functional ULOQ	Serum/EDTA Plasma (4x): 200 pg/mL
LOD	0.008 pg/mL Range: 0.001 – 0.02 pg/mL
Dynamic Range	Serum/EDTA Plasma (4x): 0 - 200 pg/mL

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

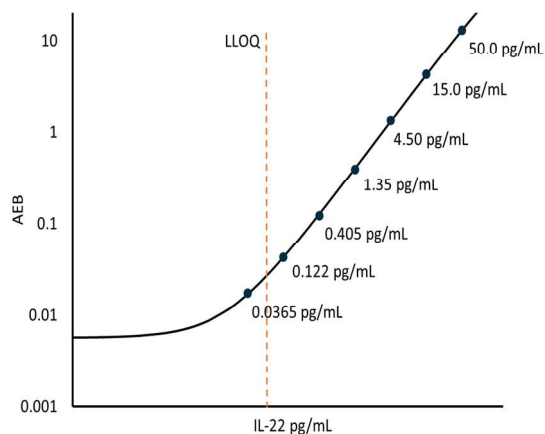
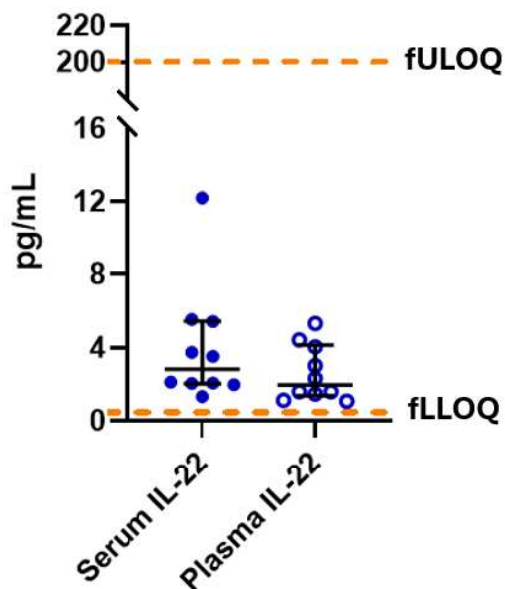


Figure 1. Example calibrator curve.

Endogenous Sample Reading: Healthy donor EDTA plasma (n=10), and serum (n=10) concentrations (pg/mL) were measured using the IL-22 Advantage PLUS kit on HD-X. Bars depict median with interquartile range. Orange line represents functional LLOQ and ULOQ.



Precision: Measurements of 2 calibrator-based controls, 2 endogenous plasma samples and 4 spiked plasma samples. Triplicate measurements were made for 3 runs each for 2 reagent lot across 2 instruments (12 runs total, 36 measurements). All samples were diluted at the recommended MRD for the sample matrix.

IL-22					
Sample	Mean (pg/mL)	Within Run CV	Between Run CV	Between Lot CV	Between Instr CV
Control 1	1.66	5.0%	7.0%	2.5%	4.0%
Control 2	103	4.0%	5.6%	2.3%	0.6%
Endogenous Plasma 1	2.21	3.3%	5.4%	5.3%	2.2%
Spiked Plasma 1	8.20	3.7%	5.4%	0.8%	3.3%
Spiked Plasma 1	45.5	4.7%	13.3%	3.8%	5.8%
Endogenous Plasma 2	5.21	2.9%	9.9%	9.6%	0.2%
Spiked Plasma 3	10.8	2.5%	9.9%	8.0%	0.1%
Spiked Plasma 4	53.3	3.7%	11.3%	5.7%	0.7%

IL-22				
Sample Type	Mean (pg/mL)	Median (pg/mL)	% Above LOD	% Above LLOQ
Serum	4	2.82	100%	100%
EDTA Plasma	2.6	1.96	100%	100%

Spike and Recovery: 2 serum and 2 EDTA plasma samples were spiked at high and low concentrations of IL-22 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 serially diluted 2x with sample diluent. Total dilution of each sample ranged from 4x to 32x.

IL-22	
Spike and Recovery Serum	Mean: 71.0% Range: 63.3 – 75.3%
Spike and Recovery EDTA Plasma	Mean: 69.3% Range: 56.6 – 83.1%
Dilution Linearity Serum (4X-32X)	Mean: 116.1% Range: 105.4 – 133.1%
Dilution Linearity EDTA Plasma (4X-32X)	Mean: 117.2% Range: 97.8 – 132.4%

The Simoa IL-22 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.