

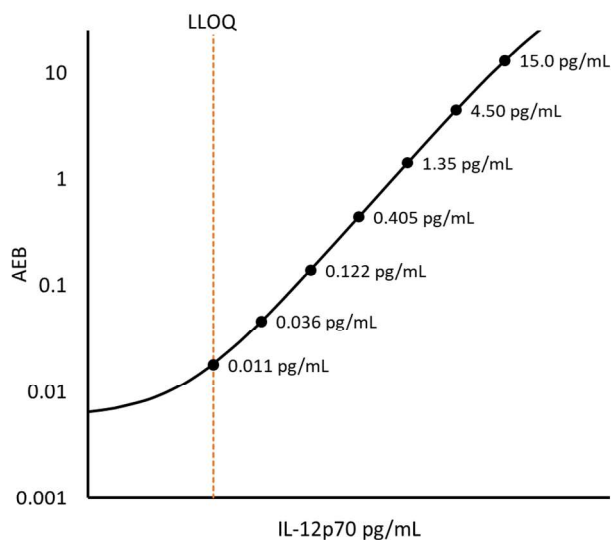
**Description**

This data sheet summarizes data from the analytical validation performed at Quanterix to characterize performance of the Simoa® IL-12p70 Advantage PLUS kit on the HD-X platform.

**IL-12p70**

Interleukin-12p70 (IL-12 p70) is a disulfide-linked heterodimeric 70-kDa cytokine composed of a 197 amino acid 35-kDa (p35) subunit and a 306 amino acid 40-kDa (p40) subunit. It is naturally produced by dendritic cells, macrophages and human B-lymphoblastoid cells in response to antigenic stimulation. IL-12p70 stimulates growth and function of T cells, production of interferon-gamma (IFN-γ) and tumor necrosis factor-alpha (TNF-α) from T cells and natural killer (NK) cells, and reduces IL-4-mediated suppression of IFN-γ. IL-12p70 has been reported to be associated with autoimmune and inflammatory conditions. Increased IL-12p70 plasma levels may also be detected in patients with neurological disorders such as multiple sclerosis.

**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.

**Minimum Required Dilution (MRD)**

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

See Kit Instruction for details.

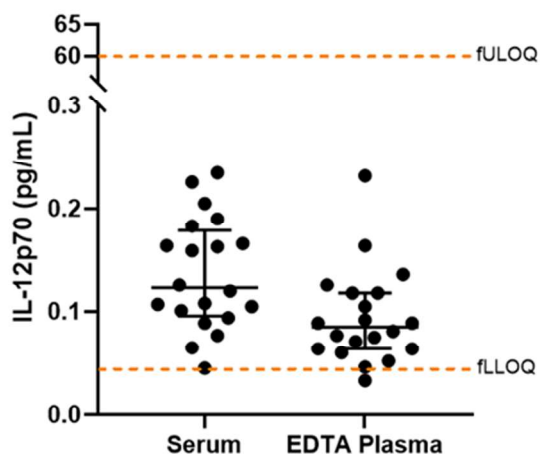
**Lower Limit of Quantification (LLOQ):** The analytical LLOQ was set at the lowest concentration that read back within 80 – 120% of the expected value with a CV ≤ 20%. The functional LLOQ (fLLOQ) values below are for serum and EDTA plasma and represent the analytical LLOQ multiplied by the dilution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

**Limit of Detection (LOD):** 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-12p70	
Analytical LLOQ	0.011 pg/mL Pooled CV: 18.1% Recovery: 97.2%
Functional LLOQ	Serum/EDTA Plasma (4x): 0.044 pg/mL
LOD	0.003 pg/mL Range: 0.001 – 0.006 pg/mL
Dynamic Range	Serum/EDTA Plasma (4x): 0 - 60 pg/mL

**Endogenous Sample Reading:** Healthy donor unmatched serum (n=20), and EDTA plasma (n=20) concentrations (pg/mL) were measured using the IL-12p70 Advantage PLUS kit on HD-X. Bars depict median with interquartile range. Orange lines represent functional LLOQ and ULOQ.



IL-12p70					
Sample	Mean (pg/mL)	Within Run CV	Between Run CV	Between Lot CV	Between Instr CV
Control 1	0.742	3.3%	11.9%	2.1%	2.1%
Control 2	12.265	4.0%	9.3%	2.5%	2.5%
Serum 1	0.145	5.7%	13.9%	2.6%	2.6%
Serum 2	0.640	4.2%	13.1%	1.5%	1.5%
Serum 3	11.629	2.7%	10.0%	0.8%	0.8%
Plasma 1	0.183	5.3%	12.1%	3.2%	3.2%
Plasma 2	1.507	3.2%	9.3%	0.2%	0.2%
Plasma 3	8.093	3.5%	6.8%	3.4%	3.4%

**Spike and Recovery:** 4 serum and 4 EDTA plasma samples were spiked at high and low concentrations of IL-12p70 within the range of the 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:** 4 serum and 4 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 128x.

IL-12p70				
Sample Type	Mean (pg/mL)	Median (pg/mL)	% Above LOD	% Above LLOQ
Serum	0.136	0.123	100%	100%
EDTA Plasma	* 0.098	* 0.089	100%	95%

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

**Precision:** Measurements of 2 calibrator-based controls, 3 commercial pooled serum samples, and 3 commercial pooled plasma samples. Triplicate measurements were made for 6 runs each for 2 reagent lots across 2 instruments (12 runs total, 36 measurements). All samples were diluted at the appropriate MRD for the sample matrix.

IL-12p70	
Spike and Recovery Serum	Mean: 86.2% Range: 75.5 – 96.4%
Spike and Recovery EDTA Plasma	Mean: 84.8% Range: 71.6 – 97.9%
Dilution Linearity Serum (4x-128x)	Mean: 113.5% Range: 99.3 – 139.0%
Dilution Linearity EDTA Plasma (4x-128x)	Mean: 108.5% Range: 94.5 – 130.3%

The Simoa® IL-12p70 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.