Quanterix®
Discovery Fueled by Ultra-Sensitivity

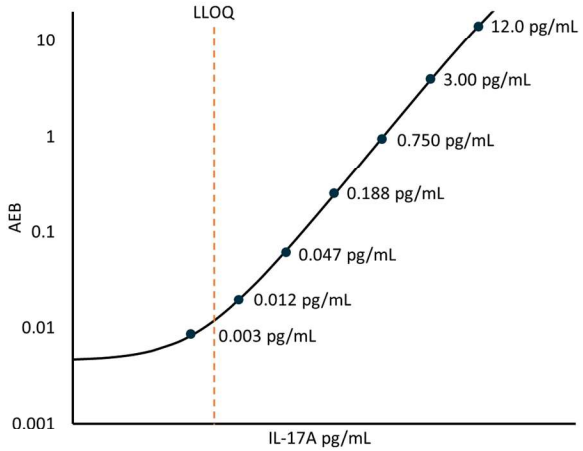
Simoa® IL-17A Advantage PLUS Kit
HD-X Data Sheet

Item 104428

Description

Interleukin 17A (IL-17A) is homo-dimeric cytokine of 155 amino acids with a molecular weight of about 35 kDa. Additional five homologous cytokines, termed IL-17B to IL-17F, have revealed. All IL-17A cytokines have a similar protein structure, and no sequence similarity to any other cytokines. These cytokines are well conserved in mammals, with significant sequence conservation between the human and mouse homologs. A major role of IL-17A is its involvement in inducing and mediating proinflammatory responses. It acts as a potent mediator in delayed-type reactions by increasing chemokine production in various tissues to recruit monocytes and neutrophils to the site of inflammation, similar to interferon gamma. The IL-17A family has been linked to many immune/autoimmune related diseases including rheumatoid arthritis, asthma, lupus, allograft rejection, anti-tumor immunity and recently psoriasis. IL-17A Advantage PLUS assay is a digital immunoassay for the quantitative determination of IL-17A in human serum and EDTA plasma.

Calibration Curve: Representative calibrator concentrations and Lower Limit of Quantification (LLOQ) depicted. For IL-17A Advantage PLUS, the reconstitution volume for calibrator concentrates may vary between kit lots, while keeping the target calibrator concentrations each level as consistent as possible: The minimum allowable concentration for Cal H is 12 pg/mL and the maximum allowable concentration for Cal B is 0.003 pg/mL.



MRD (Minimum Required Dilution)	
Diluted Sample Volume	100 µL per measurement
Serum and EDTA Plasma Dilution	1:2
Tests per kit	96

See Kit Instruction for details.

Lower Limit of Quantification (LLOQ): Triplicate measurements of serially diluted calibrator were read back on the calibration curve over 12 runs each for 2 reagent lots across 2 instruments (3 runs per lot, per instrument). 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 for each matrix represent the analytical LLOQ multiplied by the dilution factor used for the respective matrix (Table).

Limit of Detection (LOD): Calculated as 2.5 standard deviations from the mean of background signal read back on each calibration curve over 12 runs each for 2 reagent lots across 2 instruments (3 runs per lot, per instrument).

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.

Analytical LLOQ	**0.006 pg/mL** pooled CV 18% mean recovery 107%
Functional LLOQ (serum and EDTA plasma)	**0.012 pg/mL**
LOD	**0.002 pg/mL** range 0.001–0.005 pg/mL
Dynamic Range (serum and EDTA plasma)	**0 – 24 pg/mL**

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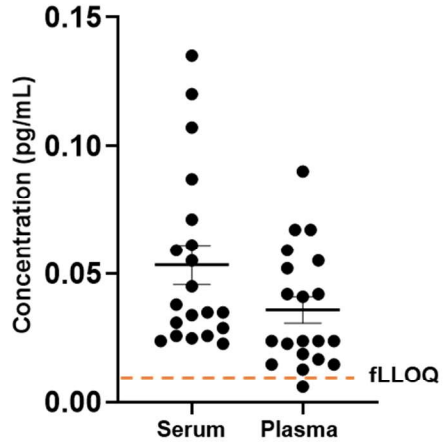
Endogenous Sample Reading: Concentrations (pg/mL) were determined for matched serum (*n*=20) and EDTA plasma (*n*=20) from normal human donors using the IL-17A Advantage PLUS kit on HD-X. Bars depict median with interquartile range. The orange lines represent functional LLOQ.



Sample Type	Mean IL-17A pg/mL	Median IL-17A pg/mL	% Above LOD	% Above LLOQ
Serum	0.053	0.037	100%	100%
Plasma	0.038	0.026	100%	96%

Precision: Measurements of 2 serum-based, 2 plasma-based panels and 2 calibrator-based controls were measured for precision. 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.

Sample	Mean (pg/mL)	Within run CV	Between run CV	Between inst CV	Between Lot CV
Control 1	0.18	4.4%	6.1%	0.1%	0.2%
Control 2	5.14	2.3%	3.5%	1.2%	3.3%
Panel 1	0.03	8.0%	9.5%	1.3%	5.5%
Panel 2	0.62	2.5%	3.8%	1.3%	0.7%
Panel 3	2.91	4.5%	6.2%	4.9%	1.4%
Panel 4	6.25	4.3%	6.0%	3.8%	3.1%

Spike and Recovery: Two normal samples for each matrix (serum and EDTA plasma) were spiked at high (1.0 pg/mL) and low (0.2 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-17A in the spiked sample and the measured concentration in unspiked sample relative to the concentration of IL-17A 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: Two human samples for each matrix (serum and EDTA plasma) were serially diluted with sample diluent through 6 levels of 2X dilutions. Each dilution series was run on the HD-X with two different lots of IL-17A Advantage PLUS assay kits with the MRD (2X) dilution applied. The total dilution of each human serum or EDTA plasma sample ranged from 2X to 8X for endogenous samples and 64X for spiked samples.

Spike and Recovery (Serum)	**Mean 76%** range 62–85%
Spike and Recovery (EDTA Plasma)	**Mean 72%** range 58–93%
Endogenous Dilution Linearity (Serum, 2X – 8X)	**Mean 114%** Range 109–118%
Endogenous Dilution Linearity (EDTA Plasma, 2X – 8X)	**Mean 113%** Range 109–126%
Spiked Dilution Linearity (Serum, 2X – 64X)	**Mean 116%** Range 103–134%
Spiked Dilution Linearity (EDTA Plasma, 2X – 64X)	**Mean 114%** Range 102–132%

The Simoa IL-17A Advantage PLUS assay kit is formulated to be compatible with HD-X platform. Verification and validation results for fully automated HD-X instrument are summarized here.

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