Quanterix™
The Science of Precision Health

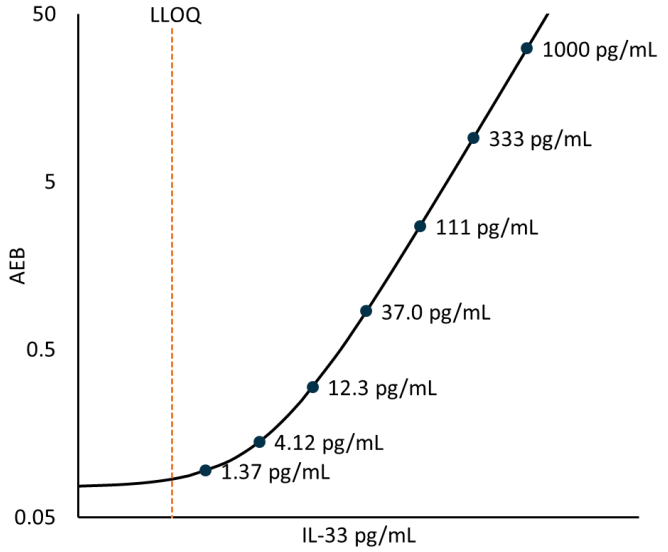
Simoa® IL-33 Discovery Kit
HD-1/HD-X Data Sheet

Item 103093

Description

IL-33 also known as NF-HEV and DVS27 is a cytokine that belongs to the IL-1 family. It is expressed by endothelial cells, fibroblasts, bronchial and epithelial cells and some immune cells including macrophages and dendritic cells. IL-33 signaling is mediated by its receptor ST2 that exists in the soluble form (sST2) and as a transmembrane receptor (ST2L). Binding to ST2L causes activation of nuclear factor κB and MAPK pathway. sST2 is thought to act as a decoy receptor attenuating the biological activity of IL-33. IL-33 mainly promotes Th2 cytokines like IL-4, IL-5 and IL-13. It is able to activate cells of the innate and adaptive immune system. Manipulation of the IL-33/sT2 pathway is being looked at as a promising method to treat or prevent various inflammatory disorders. Elevated levels of IL-33 have been observed in chronic inflammatory diseases like asthma, rheumatoid arthritis, osteoarthritis, psoriatic arthritis, SLE, UC, and Crohn's disease. Recent studies have suggested that IL-33 may play a role in Cardiovascular disorders. IL-33 inhibits the development of atherosclerotic plaques and induces the production of anti-oxidized LDL antibodies. It can also enhance eosinophilic perimyocarditis and impair heart function. The Simoa IL-33 assay detects both free and sST2-bound IL-33 as shown below.

Calibration Curve: Calibrator concentrations and Lower Limit of Quantification depicted.



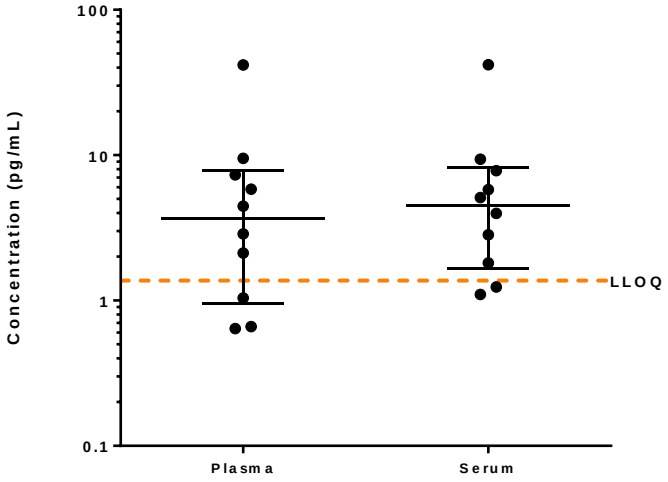
Lower Limit of Quantification (LLOQ): Triplicate measurements of serially diluted calibrator were read back on the calibration curve over 3 runs each for 1 reagent lot across 2 instruments (6 runs total).

Limit of Detection (LOD): Calculated as 2.5 standard deviations from the mean of background signal read back on each calibration curve over 3 runs each for 1 reagent lot across 2 instruments (6 runs total).

LLOQ	**0.686 pg/mL** pooled CV 16% mean recovery 108%
LOD	**0.320 pg/mL** range 0.185-0.386 pg/mL
Dynamic range	0–2000 pg/mL
Diluted Sample volume (1:2 Dilution)*	100 µL per measurement
Tests per kit	192

***See Kit Instruction for details**

Endogenous Sample Reading: Healthy donor matched EDTA plasma (n=10) and serum (n=10) samples were measured. Bars depict median with interquartile range. Orange line represents functional LLOQ.



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Sample Type	Mean IL-33 pg/mL	Median IL-33 pg/mL	% Above LOD
EDTA plasma	10.5*	5.83*	100%
Serum	9.81*	5.45*	100%

*Values below LLOQ are not included in the mean or median

Precision: Measurements of 1 endogenous serum, 2 endogenous EDTA plasma panels, and 2 calibrator-based controls. Triplicate measurements were made for 3 runs each for 1 reagent lot across 2 instruments (6 runs total, 18 measurements).

Sample	Mean (pg/mL)	Within run CV	Between run CV	Between inst CV
Control 1	28.3	3.1%	8.1%	3.9%
Control 2	409	3.7%	3.7%	4.9%
Panel 1	17.4	6.0%	7.3%	2.7%
Panel 2	5.39	9.5%	5.0%	2.3%
Panel 3	10.2	5.1%	4.4%	0.6%

Spike and Recovery: 2 EDTA plasma and 2 serum samples were spiked at high and low concentrations within the range of the assay.

Dilution Linearity: 1 spiked EDTA plasma, 1 endogenous EDTA plasma, 1 spiked serum, and 1 endogenous serum sample were diluted serially from MRD (2x) to 16x with sample diluent.

Spike and Recovery (Serum/Plasma)	**75%** Range 65-84%
Plasma Dilution Linearity (16x)	**Mean = 117%** Range: 72-132%
Serum Dilution Linearity (16x)	**Mean = 121%** Range: 108-135%

Specificity: Two normal serum samples and one spiked serum sample were pre-incubated with 25x IL-33 capture beads. Capture beads were removed and samples were run at MRD in the assay. Average knock-down relative to control without capture bead pre-incubation was **96%**.

Soluble IL-33 receptor (sST2) binding: 1 serum, 2 plasma, and one stimulated plasma were compared with and without the addition of 10 ng/mL IL-33 receptor sST2. No significant difference in sample reading was observed between the two conditions suggesting that the IL-33 assay recognizes both free and sST2-bound forms of the target.

Sample	Unspiked(pg/mL)	+ 10ng/mL sST2 (pg/mL)	Bias
Serum	1.82	1.73	-5.0%
EDTA Plasma 1	2.66	2.47	-7.0%
EDTA Plasma 2	0.991	0.993	0.3%
Stimulated plasma 1	1.36	0.966	-28.7%

The Simoa IL-33 Discovery assay kit is formulated for use on the SR-X®, HD-1, or HD-X® platform. Data in this document was obtained from runs on the HD-1 platform unless otherwise noted. Some differences in performance claims between SR-X and HD-1/HD-X may be observed when comparing datasheets for these platforms. This may be due to experiments run at different time-points with different reagent lots and different samples, or it may be due to minor differences in antibody and analyte behavior in the different assay formats.