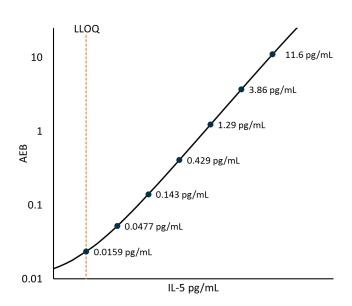
## Simoa® IL-5 Advantage Kit HD-1/HD-X Data Sheet Item 102860

## Description

Interleukin 5 (IL-5) is a cytokine with a length of 115 amino acids and a molecular weight of 15.2 kDa that is derived from T-cells with hematopoietic functions predominantly associated with antigen-induced eosinophilia. IL-5 induces differentiation of B-cells to immunoglobulin secreting cells and is an important factor in growth, differentiation and activation of eosinophils. IL-5, GM-CSF and IL-3 comprise the  $\beta$ -common ( $\beta$ c) cytokine family, so named because the receptors share a common  $\beta$  chain complexed with cytokine-specific  $\alpha$  chains. IL-5 and IL-5R are the targets of therapeutic antibodies for treatment of eosinophilic asthma and are involved in type 2 inflammation in the mucosal allergic reaction to grass pollen. Activation of the IL-3/IL-5/GM-CSF receptors results in rapid activation of the JAK/STAT pathway.

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



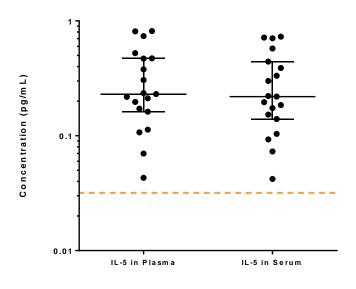
**Lower Limit of Quantification (LLOQ):** Triplicate measurements of serially diluted calibrator were read back on the calibration curve over 14 runs total over 2 reagent lots across 3 instruments.

**Limit of Detection (LOD):** Calculated as 2.5 standard deviations from the mean of background signal read back on each calibration curve over 14 runs total over 2 reagent lots across 3 instruments.

LLOQ	<b>0.0165 pg/mL</b> pooled CV 18% mean recovery 98%
LOD	<b>0.0041 pg/mL</b> range 0.0017-0.0105 pg/mL
Dynamic range (serum and plasma)	0 - ~12 pg/mL
Diluted Sample volume*	152 μL
Tests per kit	per measurement 96
rests per kit	30

<sup>\*</sup>See Kit Instruction for details

**Endogenous Sample Reading:** Healthy donor matched EDTA plasma (n=19), and serum (n=19) were measured. Bars depict median with interquartile range. Orange line represents functional LLOQ.



Sample Type	Mean IL-5 pg/mL	Median IL-5 pg/mL	% Above LOD
Serum	0.31	0.22	100%
Plasma	0.33	0.23	100%

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**Precision:** Measurements of 3 serum-based panels and 2 calibrator-based controls. Triplicate measurements were made for 14 runs total over 2 reagent lots across 3 instruments.

Sample	Mean (pg/mL)	Within run CV	Between run CV	Between inst CV	Between Lot CV
Control 1	0.32	4.1%	13.0%	7.9%	14.4%
Control 2	5.30	4.6%	14.3%	5.9%	15.8%
Panel 1	0.56	3.8%	8.3%	4.2%	4.6%
Panel 2	1.30	4.0%	8.1%	6.2%	5.9%
Panel 3	5.03	6.5%	10.4%	5.5%	10.0%

**Spike and Recovery:** 2 serum and 2 EDTA plasma samples were spiked at high and low concentrations within the range of the assay and analyzed on HD-1.

**Dilution Linearity:** 2 EDTA plasma (1 endogenous and 1 spiked) and 2 serum samples (1 endogenous and 1 spiked) were diluted 2x serially from MRD (2x) to 32x (endogenous) or 256x (spiked).

Spike and Recovery	Mean = 85%
(Serum/Plasma)	Range: 71-90%
<b>Endogenous Dilution</b>	Mean = 113%
Linearity (32x)	Range: 98-130%
Spiked Dilution	Mean = 113%
Linearity (256x)	Range: 95–139%

The Simoa IL-5 Advantage 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 the 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 may be due to minor differences in antibody and analyte behavior in the different assay formats.