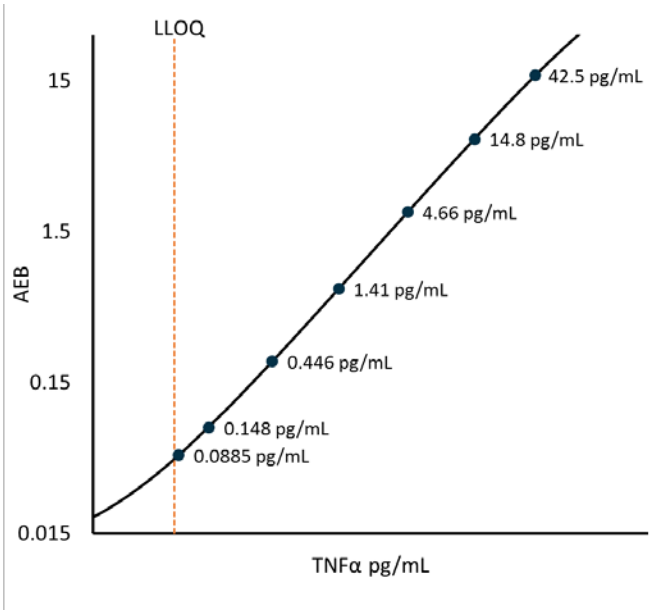
Simoa™ Cytokine 3-Plex A (TNFα, IL-6, IL-10) Kit
SR-X™ Data Sheet

Item 101160

Description

Human tumor necrosis factor alpha (TNFα) is a homotrimeric transmembrane protein that functions as a proinflammatory cytokine. It is produced mainly by macrophages but also by a variety of other cell types, including monocytes, neutrophils, and T-cells. The involvement of TNFα in several signal transduction pathways links the protein to such diverse functions as acute inflammation, apoptosis, septic shock, cellular proliferation, and differentiation. Human TNFα is a non-glycosylated protein of 157 amino acids, with a molecular weight of approximately 17,000 daltons. The clinical relevance of TNFα stems from its association with numerous disease states including rheumatoid arthritis, cancer, cachexia, and Crohn's disease.

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



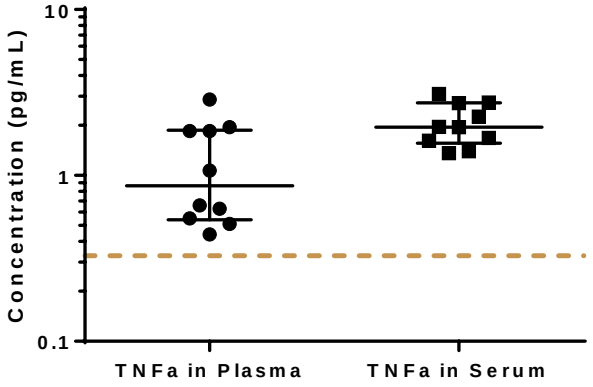
Lower Limit of Quantification (LLOQ): Triplicate measurements of serially diluted calibrator were read back on the calibration curve over 6 runs each for 1 reagent lot across 3 instruments (6 runs total).

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

LLOQ	**0.082 pg/mL** pooled CV 16% mean recovery 90%
LOD	**0.0209 pg/mL** range 0.0024-0.0832 pg/mL
Dynamic range (serum and plasma)	0 - ~188 pg/mL
Diluted Sample volume*	100 µL per measurement
Tests per kit	96

***See Kit Instruction for details**

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



Sample Type	Mean TNFα pg/mL	Median TNFα pg/mL	% Above LOD
Serum	1.24	0.866	100%
Plasma	2.08	1.96	100%

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Assay designed by Rob

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

	Mean (pg/mL)	Within run CV	Between run CV	Between inst CV
Control 1	0.480	4.4%	2.6%	1.7%
Control 2	11.90	8.2%	4.8%	2.7%
Panel 1	0.338	5.6%	6.7%	4.7%
Panel 2	0.422	8.6%	2.0%	2.4%
Panel 3	4.84	9.0%	9.9%	9.9%
Panel 4*	1.54	7.8%	8.5%	10.3%

*Plasma-based panel

Spike and Recovery: 2 serum and 2 EDTA plasma samples were spiked at low concentrations within the range of the assay and analyzed on SR-X.

Dilution Linearity: 1 spiked EDTA plasma and 1 spiked serum sample were diluted 2X serially from MRD (4x) to 256x with Sample Diluent.

Spike and Recovery (Serum/Plasma)	**Mean = 69.4%** Range: 52-81%
Dilution Linearity (256x)	**Mean = 121%** Range: 101–138%

The Simoa Cytokine 3-Plex A assay kit is formulated for use on either the SR-X or HD-1 platform. Data in this document was obtained from runs on the SR-X platform unless otherwise noted. Some differences in performance claims between the HD-1 and SR-X may be observed when comparing datasheets for the two 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.

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Simoa™ Cytokine 3-Plex A (TNFα, IL-6, IL-10) Kit

SR-X™ Data Sheet

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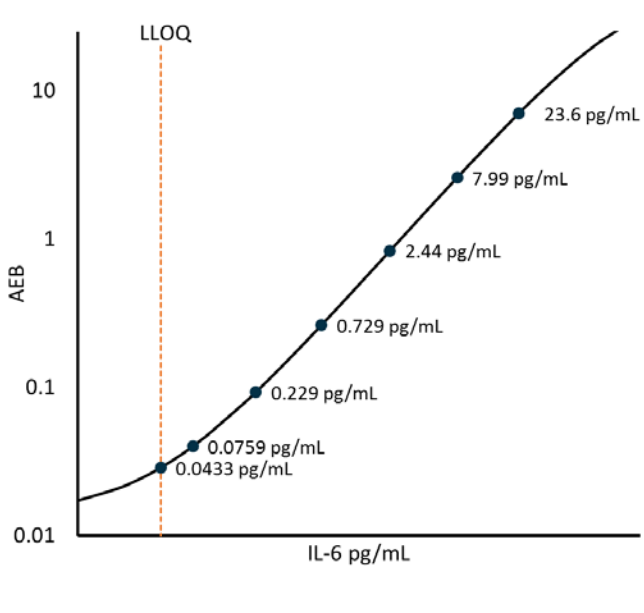
Description

Interleukin 6 (IL-6) is an alpha-helical cytokine with a wide variety of biological functions, including inducement of acute phase reactions, inflammation, hematopoiesis, bone metabolism, and cancer progression. It is secreted by multiple cell types as a 22k-28k dalton phosphorylated and variably glycosylated molecule. Mature human IL-6 is 183 amino acids (aa) in length and shares 41% aa sequence identity with mouse and rat IL-6. IL-6 is secreted by T cells and macrophages to induce immune responses following tissue trauma leading to inflammation. IL-6 also acts as an anti-inflammatory myokine, secreted by muscles during contraction after which it acts to increase breakdown of fats and improve insulin resistance. Because of its role in inducing inflammation and auto-immune response, there is interest in developing anti-IL-6 agents as potential therapies against various diseases, including rheumatoid arthritis and cancer.

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



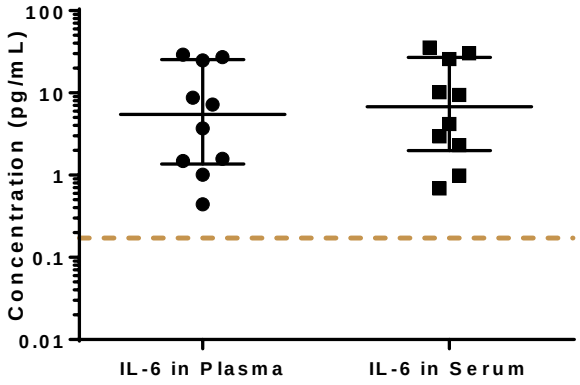
Lower Limit of Quantification (LLOQ): Triplicate measurements of serially diluted calibrator were read back on the calibration curve over 6 runs each for 1 reagent lot across 3 instruments (6 runs total).

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

LLOQ	**0.043 pg/mL** pooled CV 21% mean recovery 118%
LOD	**0.0142 pg/mL** range 0.0021-0.0374 pg/mL
Dynamic range (serum and plasma)	0 - ~80 pg/mL
Diluted Sample volume*	100 µL per measurement
Tests per kit	96

***See Kit Instruction for details**

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



Sample Type	Mean IL-6 pg/mL	Median IL-6 pg/mL	% Above LOD
Serum	12.25	6.81	100%
Plasma	10.53	5.47	100%

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

Sample	Mean (pg/mL)	Within run CV	Between run CV	Between inst CV
Control 1	0.258	6.9%	4.1%	3.7%
Control 2	6.96	13.0%	4.7%	2.5%
Panel 1	14.61	8.8%	6.5%	4.6%
Panel 2	0.773	9.6%	4.9%	1.7%
Panel 3	0.339	8.7%	11.0%	11.6%
Panel 4*	0.214	9.7%	5.9%	2.7%

*Plasma-based panel

Spike and Recovery: 2 serum and 2 EDTA plasma samples were spiked at low concentrations within the range of the assay and analyzed on SR-X.

Dilution Linearity: 1 spiked EDTA plasma and 1 spiked serum sample were diluted 2X serially from MRD (4x) to 256x with Sample Diluent.

Spike and Recovery (Serum/Plasma)	**Mean = 94.7%** Range: 82-119%
Dilution Linearity (256x)	**Mean = 100.0%** Range: 89-115%

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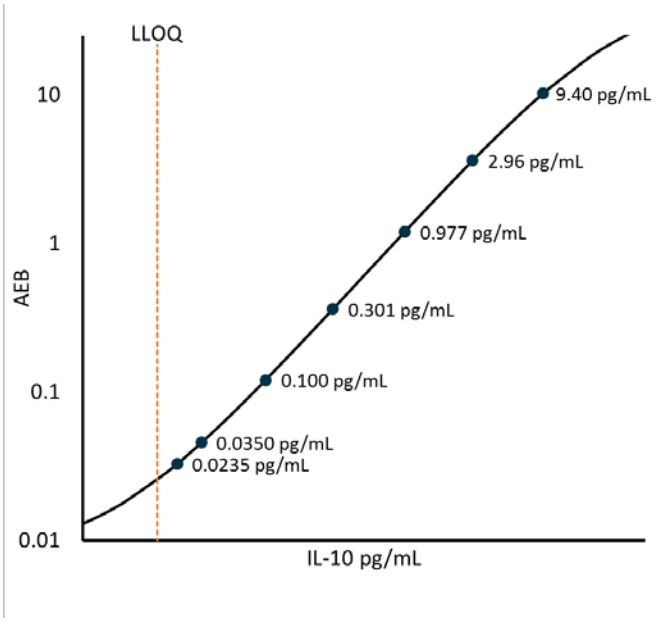
SR-X™ Data Sheet

Item 101160

Description

Interleukin 10 (IL-10) is an alpha-helical, homodimeric cytokine, each subunit composed of 178 amino acids (18 kDa). The major role of IL-10 is to act as an anti-inflammatory cytokine. It is produced primarily by monocytes, type 2 T helper cells and B cells. IL-10 is also released by cytotoxic T cells to inhibit the action of NK cells during the immune response to viral infection. It has multiple effects in immunoregulation and inflammation, including down regulation of Th1 cytokine expression, MHC class II antigens, and stimulatory molecules on macrophages. IL-10 can also inhibit synthesis of pro-inflammatory cytokines such as IFN-γ, IL-2, TNFα and GM-CSF made by macrophages and regulatory T cells. IL-10 is among cytokines secreted by muscle cells, whose elevation during physical activity suggests that exercise promotes an environment of anti-inflammatory cytokines. IL-10 has garnered interest as a potential anti-inflammatory therapeutic, but initial studies with rheumatoid arthritis have shown limited efficacy.

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



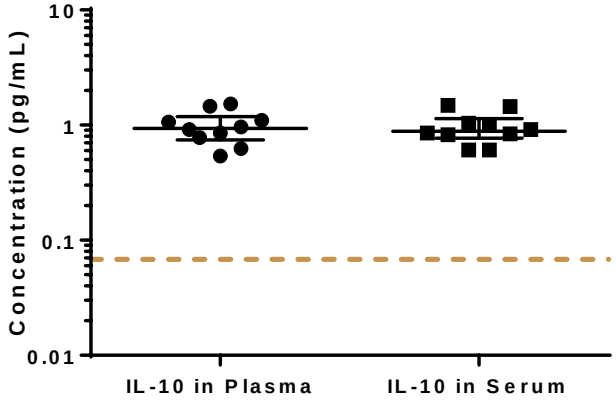
Lower Limit of Quantification (LLOQ): Triplicate measurements of serially diluted calibrator were read back on the calibration curve over 6 runs each for 1 reagent lot across 3 instruments (6 runs total).

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

LLOQ	**0.017 pg/mL** pooled CV 11% mean recovery 99%
LOD	**0.0060 pg/mL** range 0.0015-0.0190 pg/mL
Dynamic range (serum and plasma)	0 - ~40 pg/mL
Diluted Sample volume*	100 µL per measurement
Tests per kit	96

***See Kit Instruction for details**

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



Sample Type	Mean IL-10 pg/mL	Median IL-10 pg/mL	% Above LOD
Serum	0.962	0.885	100%
Plasma	0.981	0.937	100%

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

Sample	Mean (pg/mL)	Within run CV	Between run CV	Between inst CV
Control 1	0.146	3.3%	8.2%	7.9%
Control 2	3.43	8.9%	5.6%	2.5%
Panel 1	0.399	3.5%	1.8%	0.6%
Panel 2	3.04	7.6%	4.9%	2.9%
Panel 3	0.537	7.2%	9.5%	11.6%
Panel 4*	0.569	7.0%	7.9%	8.3%

*Plasma-based panel

Spike and Recovery: 2 serum and 2 EDTA plasma samples were spiked at low concentrations within the range of the assay and analyzed on SR-X.

Dilution Linearity: 1 spiked EDTA plasma and 1 spiked serum sample were diluted 2X serially from MRD (4x) to 256x with Sample Diluent.

Spike and Recovery (Serum/Plasma)	**Mean = 78.5%** Range: 72-84%
Dilution Linearity (256x)	**Mean = 123.5%** Range: 99-152%

The Simoa Cytokine 3-Plex A assay kit is formulated for use on either the SR-X or HD-1 platform. Data in this document was obtained from runs on the SR-X platform unless otherwise noted. Some differences in performance claims between the HD-1 and SR-X may be observed when comparing datasheets for the two 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.

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