

Simoa® Human Cytokine Panel 2

The Simoa Human Cytokine Panel 2 assay simultaneously measures three important cytokines in plasma and serum. The three targets are IL-1β, IL-6, and IL-10.

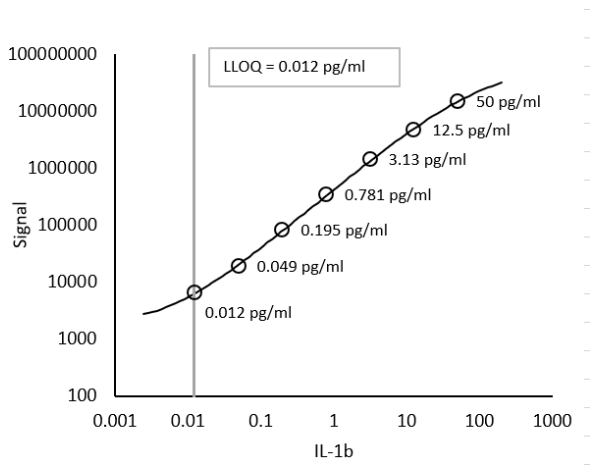
Description – IL-1β Test

Interleukin-1 beta (IL-1β, catabolin) is a 269 aa cytokine (31 kDa), produced by activated macrophages which is proteolytically processed to its active form by caspase-1. IL-1β is an important mediator of the inflammatory response involved in a variety of cellular activities including cell proliferation, differentiation, apoptosis and autoinflammatory diseases. Monocytes from patients with autoinflammatory syndromes release more processed IL-1β than cells from healthy subjects suggesting that it is involved in inflammation of these diseases. Neutralization of IL-1β results in rapid and sustained reduction in disease severity. Although some autoinflammatory diseases are due to gain-of-function mutations for caspase-1 activity, common diseases such as gout, type 2 diabetes, heart failure, recurrent pericarditis, rheumatoid arthritis, and smoldering myeloma are also responsive to IL-1β neutralization.

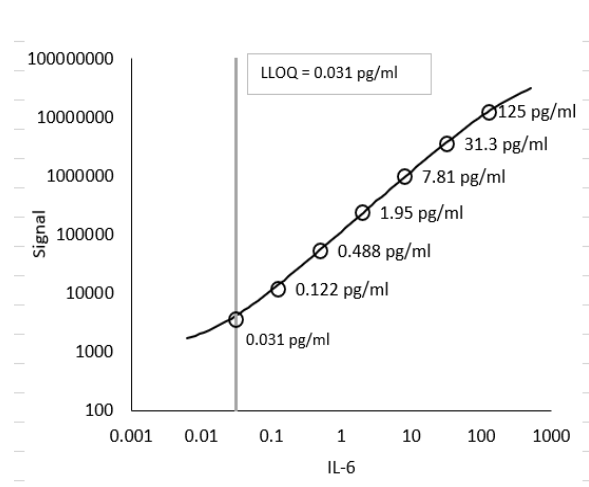
Description – IL-6 Test

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 22-28kD phosphorylated and variably glycosylated molecule. Mature human IL-6 is 183 amino acids (aa) in length. 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.



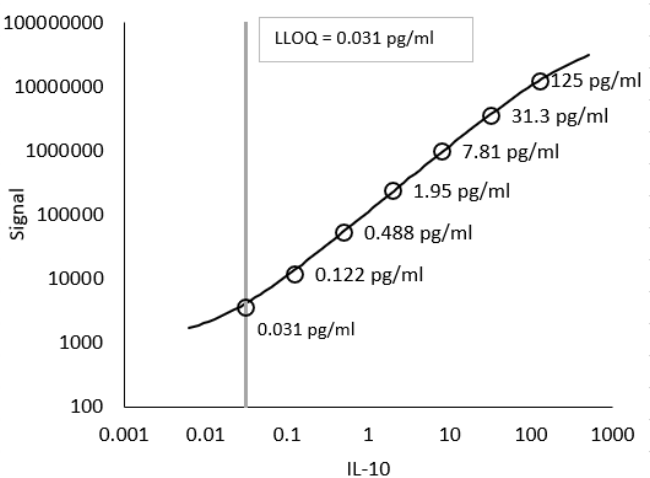
Calibration Curve: Calibrator concentrations and Lower Limit of Quantification depicted.



Description – IL-10 Test

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 natural killer 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-g, 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.



Minimum Required Dilution (MRD)

Diluted Sample Volume	50 µL per measurement
Serum and 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 6 runs each for 2 reagent lots across 2 instruments (12 runs total). The LLOQ values below are for serum and plasma.

	Analytical LLOQ	Functional LLOQ (x MRD)
IL-1β	0.012 pg/mL pooled CV 13% mean recovery 105%	0.024 pg/mL
IL-6	0.031 pg/mL pooled CV 18% mean recovery 87 %	0.062 pg/mL
IL-10	0.031 pg/mL pooled CV 10% mean recovery 81 %	0.062 pg/mL

Limit of Detection (LOD): Calculated as 2.5 standard deviations from the mean of background signal read back on each calibration curve across 2 reagent lots (12 runs total).

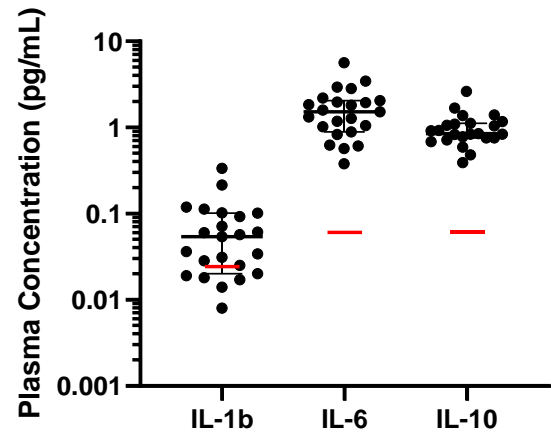
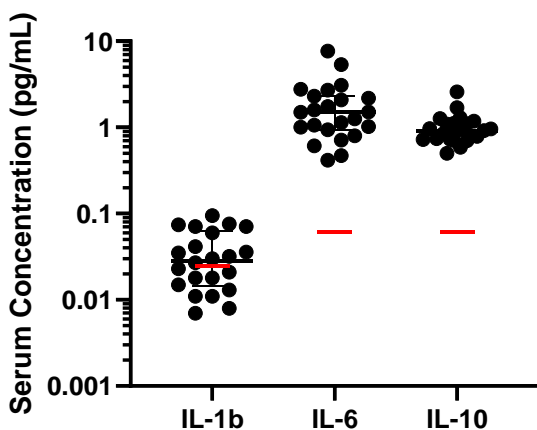
	LOD
IL-1β	0.004 pg/mL range 0.0026–0.015 pg/mL
IL-6	0.010 pg/mL range 0.0026–0.028 pg/mL
IL-10	0.016 pg/mL range 0.002–0.084 pg/mL

Assay Ranges: The upper end of the dynamic range is equal to the top calibrator concentration multiplied by MRD.

	Assay Range
IL-1β	0 - 100 pg/mL
IL-6	0 - 250 pg/mL
IL-10	0 - 250 pg/mL

Normal Range : Healthy, donor matched EDTA plasma (n=20) and serum (n=20) and unmatched EDTA plasma (n=3) and unmatched serum (n=3) were measured. Bars depict median with interquartile range. Red line represents functional LLOQ.

	Sample Type	Median Conc pg/mL	% Above LOD	% Above LLOQ
IL-1β	Serum	0.029	96%	52%
	EDTA	0.054	100%	74%
IL-6	Serum	1.508	100%	100%
	EDTA	1.521	100%	100%
IL-10	Serum	0.921	100%	100%
	EDTA	0.845	100%	100%



Precision: Measurements of 5 panels made using pooled serum-like matrix. Triplicate measurements were made for 6 runs each for 2 reagent lots across 2 instruments (12 runs total, 36 measurements).

Mean (pg/mL)	IL-1β	IL-6	IL-10
Panel 1	7.02	26.5	52.8
Panel 2	2.16	10.0	18.0
Panel 3	0.740	3.66	6.61
Panel 4	0.261	1.49	2.46
Panel 5	0.098	0.743	0.972

Inter-run CV	IL-1β	IL-6	IL-10
Panel 1	5.1%	4.2%	5.1%
Panel 2	5.2%	3.7%	4.3%
Panel 3	6.9%	5.9%	5.1%
Panel 4	7.9%	6.1%	5.4%
Panel 5	11.3%	8.6%	5.7%

Intra-run CV	IL-1β	IL-6	IL-10
Panel 1	4.5%	3.3%	4.3%
Panel 2	2.8%	2.2%	3.5%
Panel 3	2.8%	3.5%	3.2%
Panel 4	3.2%	2.7%	3.4%
Panel 5	4.3%	4.1%	3.7%

Inter-lot CV	IL-1β	IL-6	IL-10
Panel 1	0.9%	1.9%	0.9%
Panel 2	0.3%	1.5%	0.9%
Panel 3	6.0%	1.4%	1.8%
Panel 4	4.4%	1.3%	1.3%
Panel 5	7.0%	0.2%	0.3%

Between Instrument CV	IL-1β	IL-6	IL-10
Panel 1	1.7%	0.5%	0.5%
Panel 2	1.7%	1.7%	0.3%
Panel 3	0.3%	3.6%	2.2%
Panel 4	2.1%	3.1%	2.9%
Panel 5	1.7%	4.3%	2.0%

Dilution Linearity: 2 EDTA plasma and 2 serum samples were spiked with a panel that was stimulated with PMA & LPS and diluted 2x serially from MRD (2x) to 256x with Sample Diluent.

IL-1β (128X)	Mean 112% range 100%-118%
IL-6 (32X)	Mean 101% range 88%-112%
IL-10 (8X)	Mean 81% range 69%-91%

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 SP-X.

IL-1β	Grand Mean 83% Serum Mean 77% Serum Range: 67-85% Plasma Mean 89% Plasma Range: 80%-99% Grand Mean Range: 67%-99%
IL-6	Grand Mean 61% Serum Mean 42% Serum Range: 29%-55% Plasma Mean 79% Plasma Range: 72%-89% Grand Mean Range: 29%-89%
IL-10	Grand Mean 86% Serum Mean 77% Serum Range: 68%-88% Plasma Mean 95% Plasma Range: 90%-102% Grand Mean Range: 68%-102%