Simoa® Planar Array Rat Cytokine Panel 1

SP-X™ Data Sheet Item 85-0015

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Simoa Planar Array Rat Cytokine Panel 1 is a multiplex immunoassay designed for the Quanterix SP-X™ Imaging and Analysis system, which simultaneously measures seven important cytokines in rat serum and plasma. The seven soluble proteins measured by the assay include Interferon gamma (IFNγ), IL-1β, IL-2, IL-6, IL-10, GROα/KC, and Tumor Necrosis Factor alpha (TNFα).

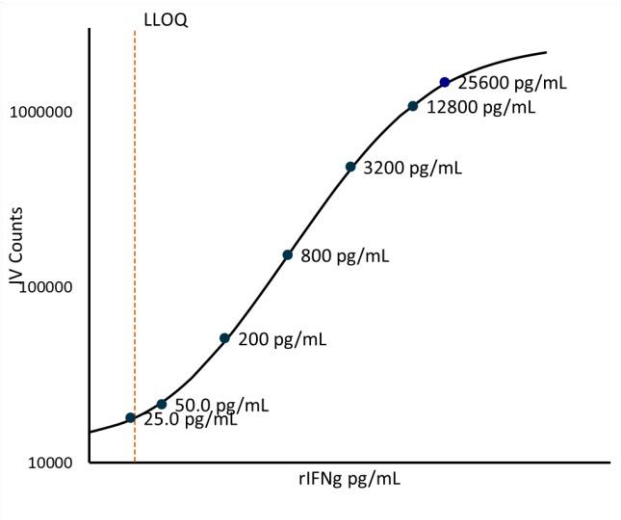
Description – rIFN-γ Test

Interferon-gamma (IFNγ) in mature form is a dimeric cytokine. Murine and human IFNγ show approximately 40% sequence homology at the protein level. IFNγ is expressed by Th1 cells, Tc cells, dendritic cells and natural killer cells, especially under inflammatory conditions, and functions via binding to a heterodimeric receptor IFNγR. It plays a key role in host defense through development and activation of Th1 cells. IFN-γ stimulates the antimicrobial and antitumor activity of macrophages, NK cells and neutrophils, and also functions as an anti-inflammatory mediator by promoting the development of regulatory T cells and inhibiting Th17 cell differentiation, and exhibits antiviral, antiproliferative, and apoptotic effects. IFNγ is an attractive drug target for immuno-regulatory diseases.

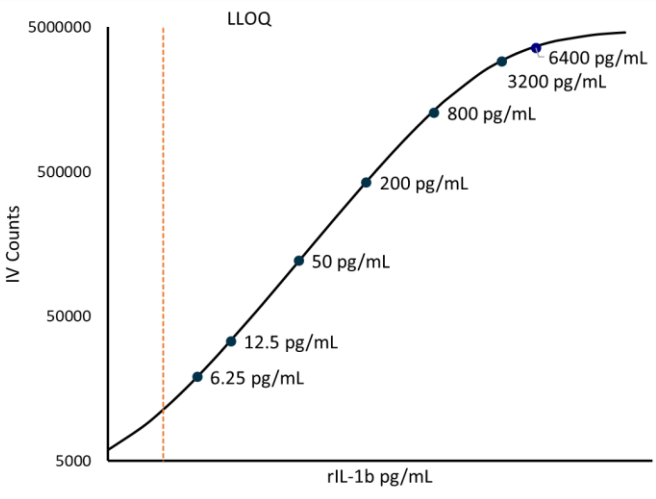
Description – rIL-β Test

Interleukin-1 beta (IL-1β, catabolin) is produced by activated macrophages as a proprotein 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 smouldering myeloma are also responsive to IL-1β neutralization.

Rat IFN-γ Curve: Calibrator concentrations, and Lower Limit of Quantification depicted.



Rat IL-1β Curve: Calibrator concentrations, and Lower Limit of Quantification depicted.



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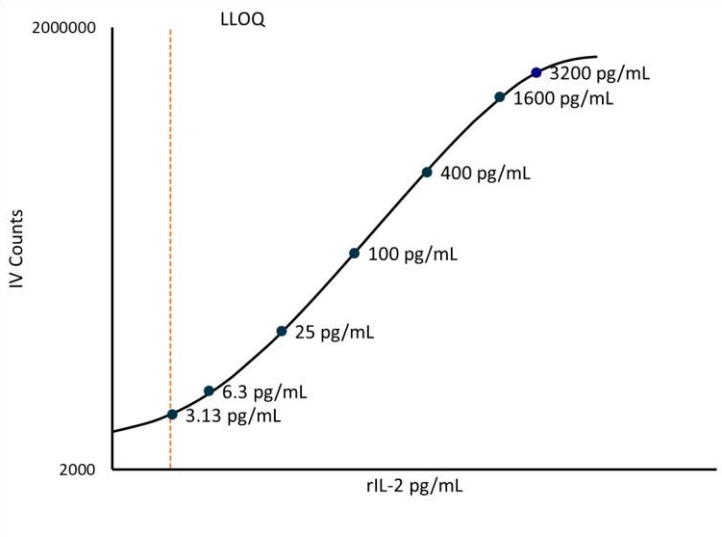
Description – rIL-2 Test

Interleukin 2 (IL-2) plays a primary role in regulation of activities of lymphocytes that are responsible for immunity. During infection, the binding of antigens to T cell receptors triggers secretion of IL-2 and expression of IL-2 receptors (IL-2R), promoting the growth, proliferation, and differentiation of CD8+ T cells into effector cells and memory cells. IL-2 is also responsible for discrimination between foreign ("non-self") and "self", and as such is a target of immunosuppressive regimens which inhibit the production of IL-2 by antigen-activated T cells and block IL-2R signaling, preventing the clonal expansion and function of antigen-selected T cells.

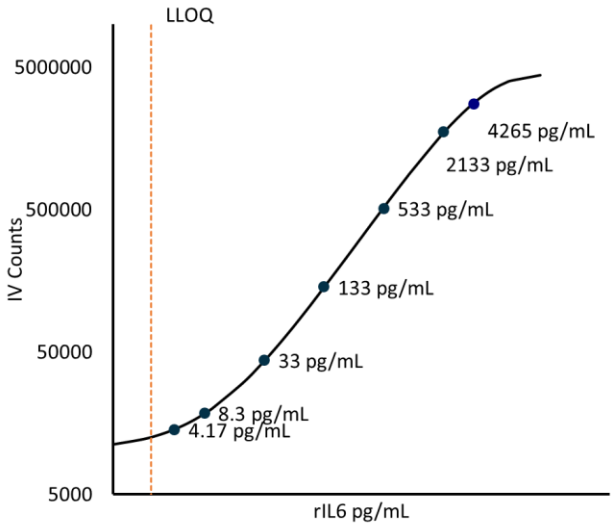
Description – rIL-6 Test

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. 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.

Rat IL-2 Curve: Calibrator concentrations, and Lower Limit of Quantification depicted.



Rat IL-6 Curve: Calibrator concentrations, and Lower Limit of Quantification depicted.



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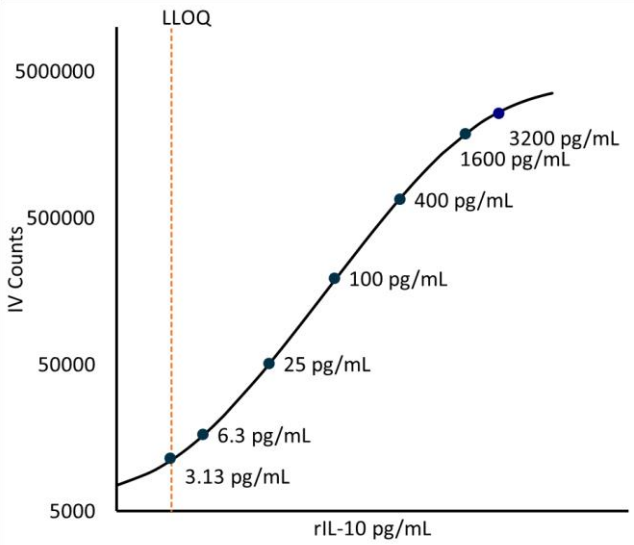
Description – rIL-10 TEST

Interleukin 10 (IL-10) is an alpha-helical, homodimeric cytokine, with 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, TNFa and GM-CSF made by macrophages and regulatory T cells. IL-10 is among the 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.

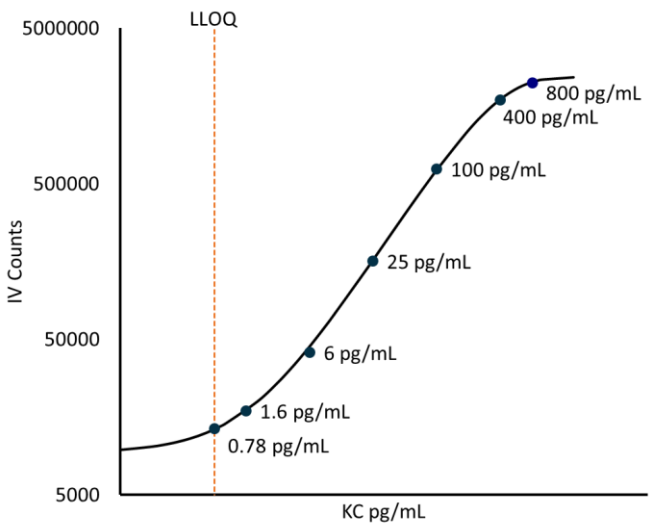
Rat IL-10 Curve: Calibrator concentrations, and Lower Limit of Quantification depicted.



Description – rGROα/KC Test

Rat GROα/KC, also known as CXCL1, CINC-1, NAP-3, is a member of the CXC family of chemokines. It is expressed by mast cells, macrophages, neutrophils and epithelial cells. It has a neutrophil chemoattractant activity. The mouse and rat GROα/KC respectively share 57% and 59% amino acid sequence identity with the human protein.

rGROα/KC Curve: Calibrator concentrations, and Lower Limit of Quantification depicted.



Description – rTNFα Test

Tumor Necrosis Factor alpha (TNFα) is a polypeptide cytokine produced by monocytes and macrophages. 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. The clinical relevance of TNFα is from its association with numerous disease states including rheumatoid arthritis, cancer, cachexia, and Crohn's disease.

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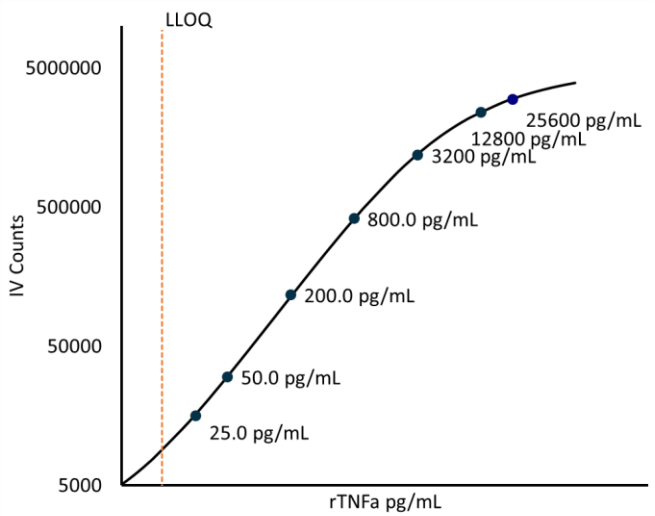
rTNFα Curve: Calibrator concentrations, and Lower Limit of Quantification depicted.



Minimum Required Dilution (MRD) and Tests per kit

Diluted Sample volume (1:2 Dilution) *	50 µL per measurement
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 (13 runs total). Analytical Lower Limit of Quantification (LLOQ) is the lowest calibration standard with back-calculated concentration pooled CV <20% and relative error <25%.

	Analytical LLOQ	Functional LLOQ (x MRD)
rIFNγ	**27.3 pg/mL** pooled CV 9.4% mean recovery 109%	**54.5 pg/mL**
rIL-1β	**3.1 pg/mL** pooled CV 12.8% mean recovery 100%	**6.3 pg/mL**
rIL-2	**3.0 pg/mL** pooled CV 6.6% mean recovery 96%	**6.0 pg/mL**
rIL-6	**2.4 pg/mL** pooled CV 10.6 % mean recovery 117%	**4.8 pg/mL**
rIL-10	**3.2 pg/mL** pooled CV 6.1 % mean recovery 102%	**6.4 pg/mL**
rGROα/KC	**0.8 pg/mL** pooled CV 4.4 % mean recovery 100%	**1.6 pg/mL**
rTNFα	**12.1 pg/mL** pooled CV 10.4 % mean recovery 97%	**24.2 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 (8 runs total).

	LOD
rIFNγ	**10.48 pg/mL** range 0.60 – 22.37
rIL-1β	**0.66 pg/mL** range 0.14 – 1.26
rIL-2	**0.91 pg/mL** range 0.04 – 2.82
rIL-6	**1.52 pg/mL** range 0.15 – 2.40
rIL-10	**0.36 pg/mL** range 0.04 – 0.99
rGROα/KC	**0.20 pg/mL** range 0.02 – 0.44
rTNFα	**1.67 pg/mL** range 0.09 – 3.96

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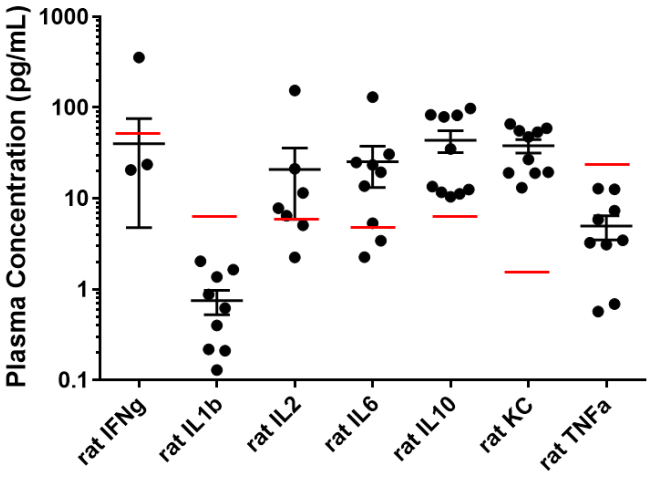
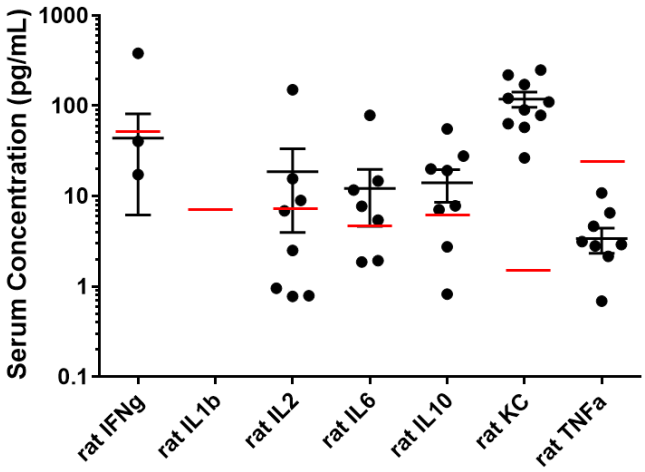
Assay Ranges: The upper end of the dynamic range is equal to the top calibrator concentration multiplied by MRD.

	Assay Range
rIFNγ	0 – 51,200 pg/mL
rIL-1β	0 – 12,800 pg/mL
rIL2	0 – 6,400 pg/mL
rIL-6	0 – 8,530 pg/mL
rIL-10	0 – 6,400 pg/mL
rGROα/KC	0 – 1,600 pg/mL
rTNFα	0 – 51,200 pg/mL



Endogenous Serum and Plasma Readings: Healthy donor matched EDTA plasma (n=10) and serum (n=10) samples from Sprague Dawley rats were measured. Bars depict median with interquartile range. Red lines represent functional LLOQ.



	Sample Type	Median Conc pg/mL	% Above LOD	% Above LLOQ
rIFNγ	Serum	43.9	20%	10%
	EDTA	40.2	30%	10%
rIL-1β	Serum	under	0%	0%
	EDTA	0.8	30%	0%
rIL-2	Serum	18.7	50%	40%
	EDTA	20.9	70%	50%
rIL-6	Serum	12.2	50%	50%
	EDTA	25.4	80%	70%
rIL-10	Serum	14.1	80%	60%
	EDTA	43.8	100%	100%
rGROα/KC	Serum	119.2	100%	100%
	EDTA	38.0	100%	100%
rTNFα	Serum	3.4	30%	0%
	EDTA	5.0	60%	0%

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Dilution Linearity: Three spiked serum and 3 spiked plasma samples were diluted 2x according to the MRD, and then serially diluted 2x with Sample Diluent three times, for final dilution of 1:16.

rIFNγ	**Mean 129.1 %** range 101.9% – 166.0%
rIL-1β	**Mean 126.1%** range 96.0%-170.4%
rIL-2	**Mean 124.2%** range 114.1 – 131.8%
rIL-6	**Mean 116.8%** range 105.4% – 128.2%
rIL-10	**Mean 165.1%** range 98.3% – 289.2%
rGROα/KC	**Mean 128.2%** range 95.0% – 172.0%
rTNFα	**Mean 140.4%** range 87.2% – 174.6%

Spike and Recovery: Three EDTA plasma samples and 3 serum samples were spiked at high, medium and low concentrations of recombinant antigens within the range of the assay.

rIFNγ	**Mean 51%** Range 40% – 86%
rIL-1β	**Mean 31%** Range 9% – 52%
rIL-2	**Mean 59%** Range 49% – 96%
rIL-6	**Mean 60%** Range 41% – 80%
rIL-10	**Mean 46%** Range 17% – 78%
rGROα/KC	**Mean 60%** Range 36%-88%
rTNFα	**Mean 45%** Range 31% – 73%

Single-plex Correlation: Sample concentrations from eleven serum and EDTA plasma samples derived from single-plex standard curves were compared to the same samples calculated from the 7-plex standard curve. The average correlation between multi-plex and single-plex assays over the entire dynamic range is shown in the table below.

	Bias
rIFNγ	6.9%
rIL-1β	86.8%
rIL-2	31.4%
rIL-6	14.2%
rIL-10	21.2%
rGROα/KC	10.4%
rTNFα	30.9%

Parallelism: Two un-spiked serum and 2 un-spiked plasma samples were diluted 2x according to the MRD, and then serially diluted 2x with Sample Diluent two times, for final dilution of 1:8.

rIFNγ	**Mean 141.6 %** range 134.3% – 149.0%
rIL-1β	**Mean 60.3%** range 44.4% – 76.8%
rIL-2	**Mean 129.4%** range 103.6% – 152.0%
rIL-6	**Mean 127.9%** range 114.2% – 135.0%
rIL-10	**Mean 338.5%** range 153.8% – 597.9%
rGROα/KC	**Mean 205.3%** range 123.8% – 276.4%
rTNFα	**Mean 42.7%** range 42.7% – 42.7%

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Precision: Measurements of 3 calibrator-based controls. Triplicate measurements were made across 3 reagent lots (7 runs total, not adjusted for dilution).

Mean (pg/mL)	rIFNγ	rIL-1β	rIL-2	rIL-6	rIL-10	rKC	rTNFα
Control H	12,445.9	2,516.0	1,444.9	2,032.9	1,398.6	321.6	10,863.0
Control M	687.6	141.9	89.3	129.4	88.7	18.7	626.7
Control L	89.2	18.0	11.1	13.9	10.2	2.0	77.3

Inter-run CV	rIFNγ	rIL-1β	rIL-2	rIL-6	rIL-10	rKC	rTNFα
Control H	14.8%	19.3%	22.9%	10.1%	12.2%	10.6%	11.0%
Control M	15.8%	22.1%	22.2%	12.9%	12.1%	7.9%	7.5%
Control L	22.9%	19.3%	24.9%	13.8%	13.2%	14.1%	9.3%

Intra-run CV	rIFNγ	rIL-1β	rIL-2	rIL-6	rIL-10	rKC	rTNFα
Control H	6.8%	6.3%	6.2%	2.4%	6.3%	4.5%	11.2%
Control M	11.2%	7.3%	4.0%	2.7%	6.5%	4.8%	2.9%
Control L	16.9%	3.8%	11.5%	6.0%	10.3%	6.1%	5.6%

Cross-reactivity: During assay validation, cross-reactivity was assessed by testing single antigen at the concentration of the third highest calibrator in the presence of all detection antibodies (maximum 5.0% of cross reactivity), and single detection antibodies in the presence of all antigens (maximum 3.6% of cross reactivity) in assay buffer. In addition, cross-reactivity of single detection antibodies was assessed in sample matrix (maximum 28.0% of cross reactivity).

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