Simoa™ CorPlex™ Human Cytokine Panel 1

SP-X® Data Sheet Item 85-0329

CorPlex Human Cytokine 10-plex panel 1 assay (CPX)

The Simoa CorPlex Human Cytokine 10-plex Panel 1 assay (CPX) simultaneously measures ten important cytokines in blood. The ten targets are Interferon gamma (IFNγ), IL-1β, IL-4, IL-5, IL-6, IL-8, IL-10, IL-12p70, IL-22, and Tumor Necrosis Factor alpha (TNFα).

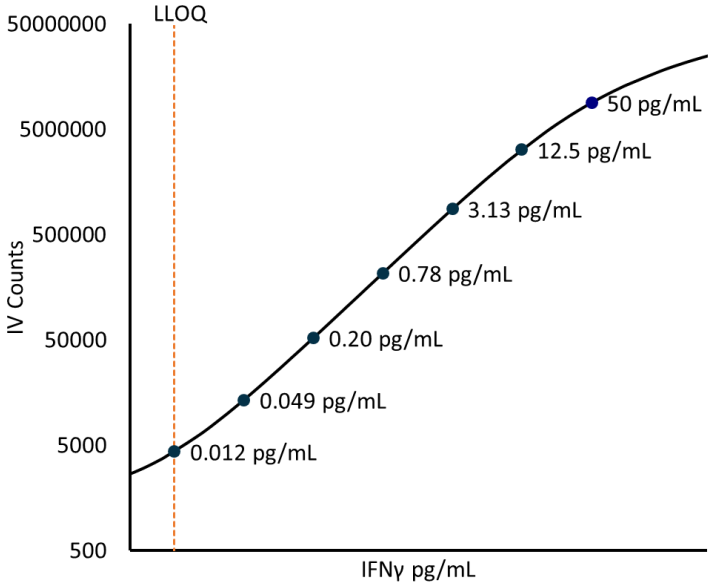
Description – IFNγ Test

Human interferon-gamma (IFNγ) in mature form is a dimeric cytokine with non-covalently subunits of 146 amino acids (20–25 kDa), that does not display significant homology with IFNα or IFNβ. 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 the receptor IFNγR. It plays a key role in host defense through development and activation of Th1 cells, chemoattraction and activation of monocytes/ macrophages, upregulation of antigen presentation molecules, and immunoglobulin class switching in B cells. IFN-γ 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.

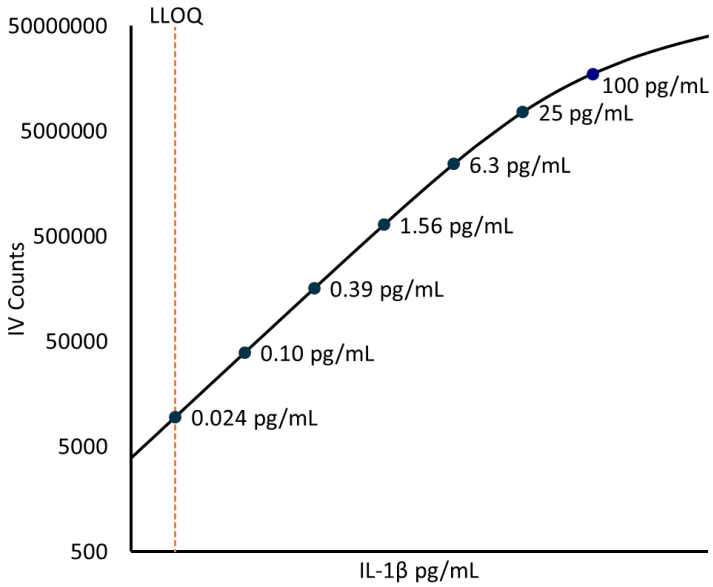
Description – IL-1β Test

Interleukin-1 beta (IL-1β, catabolin) is a 269 aa cytokine (31 kDa), 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.

IFNγ Test: Calibrator concentrations and Lower Limit of Quantification depicted.



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



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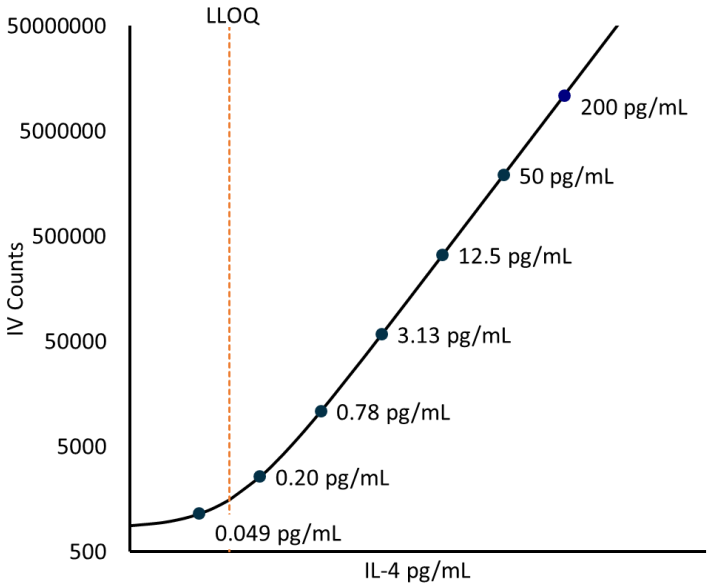
Description – IL-4 Test

Human Interleukin-4 (IL-4) is a monomeric cytokine, approximately 13-18 kDa, expressed by Th2-biased CD4+ T cells, mast cells, basophils, and eosinophils. IL-4 has a compact, globular fold (similar to other cytokines) stabilized by 3 disulphide bonds. Mature human IL-4 shares 55%, 39%, and 43% amino acid sequence identity with bovine, mouse, and rat IL-4, respectively. By binding to IL-4 receptor or receptor complex, IL-4 has many biological functions. It promotes cell proliferation, survival, and immunoglobulin class switch to IgG4 and IgE in human B cells, acquisition of the Th2 phenotype by naïve CD4+ T cells, priming and chemotaxis of mast cells, eosinophils, and basophils, and the proliferation and activation of epithelial cells. IL-4 plays an important role in the development of allergic inflammation and asthma.

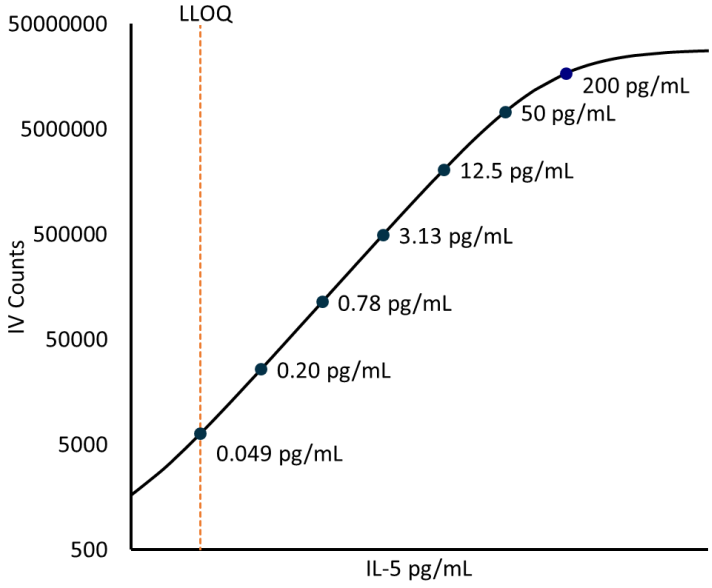
Description – IL-5 Test

Interleukin 5 (IL-5) is a cytokine which is predominantly associated with antigen-induced eosinophilia. The activation of T Helper 2 (Th2) cells leads to the production of IL-5. IL-5 consists of two identical polypeptide chains composed of 115 amino acids (molecular weight 45kDa). Several studies correlate higher levels of IL-5 with inflammatory disorders such as angioedema, eosinophilia, multiple sclerosis, persistent asthma, and cow's milk allergy in newborn infants.

IL-4 Curve: Calibrator concentrations and Lower Limit of Quantification depicted.



IL-5 Curve: Calibrator concentrations and Lower Limit of Quantification depicted.



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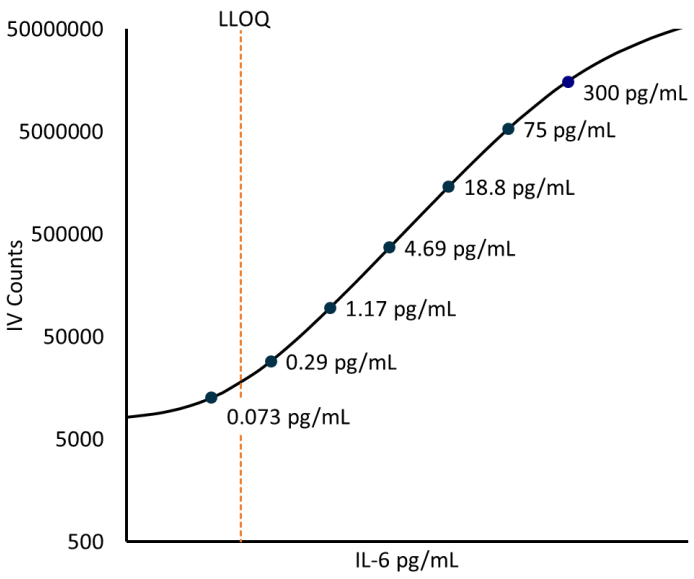
Description – IL-6 Test

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 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.

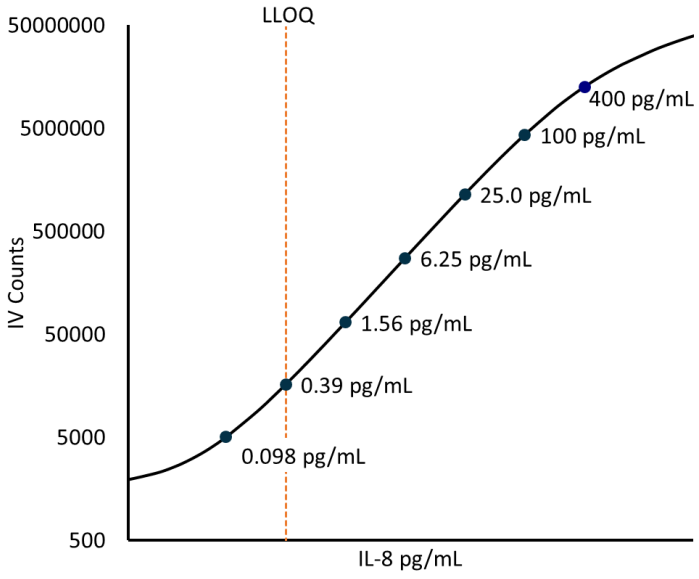
Description – IL-8 Test

Interleukin 8 (IL-8) is a cytokine of 72 amino acids (molecular weight 8 kDa) whose primary role is induction of chemotaxis in neutrophils, basophils, and T-cells, causing them to migrate to the site of infection. IL-8 also induces phagocytosis by the target cells. IL-8 is secreted by cells involved in the immune response to antigens, typically starting with macrophages, which release IL-8 to recruit other cells. Secretion of IL-8 is increased by oxidant stress, which thereby cause the recruitment of inflammatory cells, inducing a further increase in oxidant stress mediators, making it a key parameter in localized inflammation. IL-8 elevation has been associated with a range of clinical conditions, including psoriasis, chronic hepatitis C, and thyroid disease. IL-8 has recently been identified as a potential therapeutic target in inflammatory diseases.

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



IL-8 Curve: Calibrator concentrations and Lower Limit of Quantification depicted.



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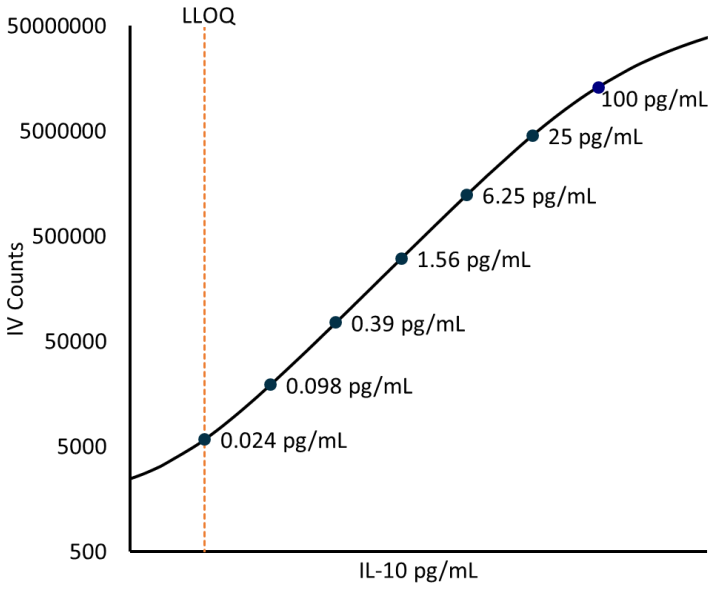
Description – IL-10 Test

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, TNFa 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.

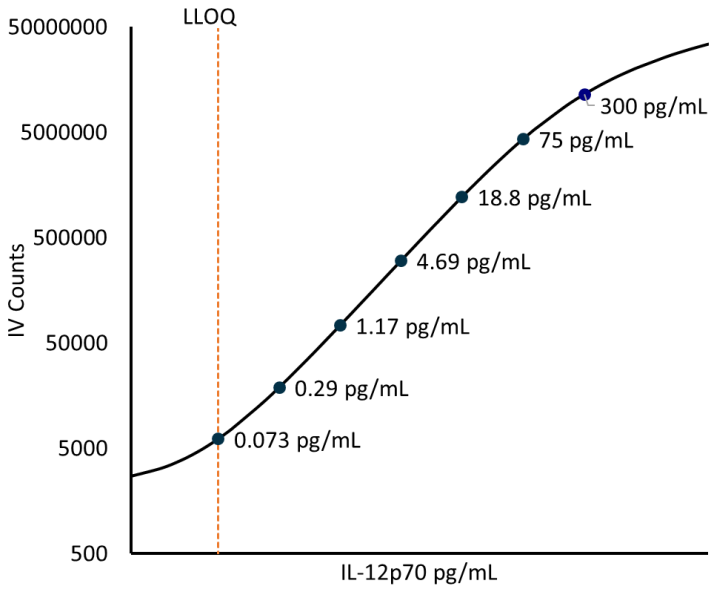
Description – IL-12p70 Test

Interleukin-12, p70 (IL-12 p70) is a disulfide-linked heterodimeric 70-kDa cytokine composed of a 197 amino acid 35-kDA (p35) subunit and a 306 amino acid 40-kDa (p40) subunit. It is naturally produced by dendritic cells, macrophages and human B-lymphoblastoid cells in response to antigenic stimulation. IL-12 stimulates growth and function of T cells, production of interferon-gamma (IFN-γ) and tumor necrosis factor-alpha (TNF-α) from T cells and natural killer (NK) cells, and reduces IL-4-mediated suppression of IFN-γ. IL-12 has been reported to be associated with autoimmune and inflammatory conditions. Increased IL-12 plasma levels may also be detected in patients with neurological disorders such as multiple sclerosis.

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



IL-12p70 Curve: Calibrator concentrations and Lower Limit of Quantification depicted.



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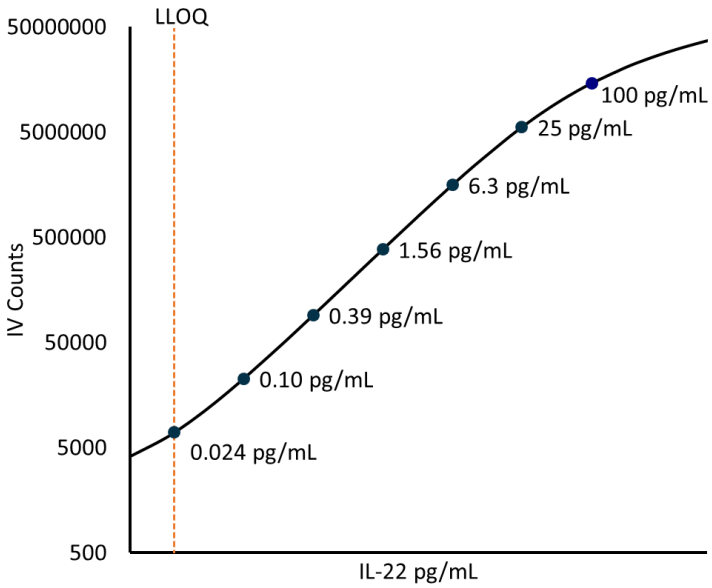
Description – IL-22 Test

IL-22 is a member of the IL-10 superfamily of cytokines. These cytokines are pleiotropic, affecting a wide range of immune functions. IL-22 is produced by Dendritic, T, and Innate Lymphoid cells and can be found in a wide range of tissues. Biological activity of IL-22 is initiated through interactions with IL-22R1 and IL-10R2, as well as IL-22BP1 and is regulated by IL-17A. IL-22 activation plays a role in the initiation and regulation of nonspecific immune response. IL-22 is associated with psoriasis; serum levels of the cytokine correlate with the severity of the disease. Emerging evidence suggests that IL-22 can play a role in other autoimmune disorders such as Inflammatory Bowel Disease, Rheumatoid Arthritis, and Multiple Sclerosis, perhaps due to its role in inflammatory responses, which are regulated by IL-17A. IL-22 has also been implicated as a Reg gene regulator promoting β-cell production in Type 1 diabetes.

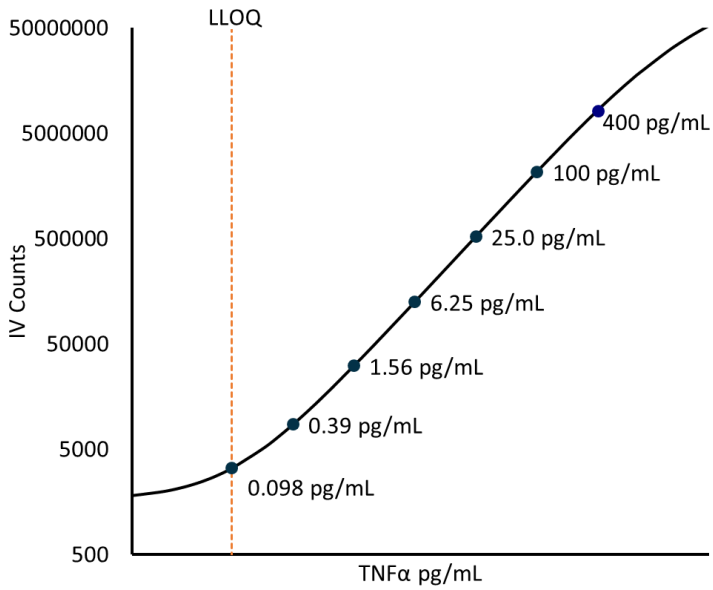
Description – TNFα Test

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.

IL-22 Curve: Calibrator concentrations and Lower Limit of Quantification depicted.



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



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Minimum Required Dilution (MRD) and Tests per kit

Diluted Sample volume (1:4 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 across 3 reagent lots (13 runs total).

	Analytical LLOQ	Functional LLOQ (x MRD)
IFNγ	**0.012 pg/mL** pooled CV 13% mean recovery 117%	**0.05 pg/mL**
IL-1β	**0.024 pg/mL** pooled CV 13% mean recovery 98%	**0.10 pg/mL**
IL-4	**0.098 pg/mL** pooled CV 19% mean recovery 104%	**0.39 pg/mL**
IL-5	**0.049 pg/mL** pooled CV 9% mean recovery 99%	**0.20 pg/mL**
IL-6	**0.148 pg/mL** pooled CV 11% mean recovery 110%	**0.59 pg/mL**
IL-8	**0.390 pg/mL** pooled CV 11% mean recovery 98%	**1.56 pg/mL**
IL-10	**0.024 pg/mL** pooled CV 10% mean recovery 110%	**0.10 pg/mL**
IL-12p70	**0.073 pg/mL** pooled CV 13% mean recovery 100%	**0.29 pg/mL**
IL-22	**0.024 pg/mL** pooled CV 10% mean recovery 104%	**0.10 pg/mL**
TNFα	**0.098 pg/mL** pooled CV 16% mean recovery 104%	**0.39 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 3 reagent lots (13 runs total).

	LOD
IFNγ	**0.007 pg/mL** range 0.002 - 0.013
IL-1β	**0.011 pg/mL** range 0.004 - 0.045
IL-4	**0.046 pg/mL** range 0.004 - 0.100
IL-5	**0.013 pg/mL** range 0.002 - 0.038
IL-6	**0.037 pg/mL** range 0.008 - 0.067
IL-8	**0.115 pg/mL** range 0.005 - 0.326
IL-10	**0.012 pg/mL** range 0.005 - 0.021
IL-12p70	**0.028 pg/mL** range 0.007 - 0.052
IL-22	**0.010 pg/mL** range 0.001 - 0.025
TNFα	**0.063 pg/mL** range 0.036 - 0.106

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

	Assay Range
IFNγ	0 - 200 pg/mL
IL-1β	0 - 400 pg/mL
IL-4	0 - 800 pg/mL
IL-5	0 - 800 pg/mL
IL-6	0 - 1200 pg/mL
IL-8	0 - 1600 pg/mL
IL-10	0 - 400 pg/mL
IL-12p70	0 - 1200 pg/mL
IL-22	0 - 400 pg/mL
TNFα	0 - 1600 pg/mL

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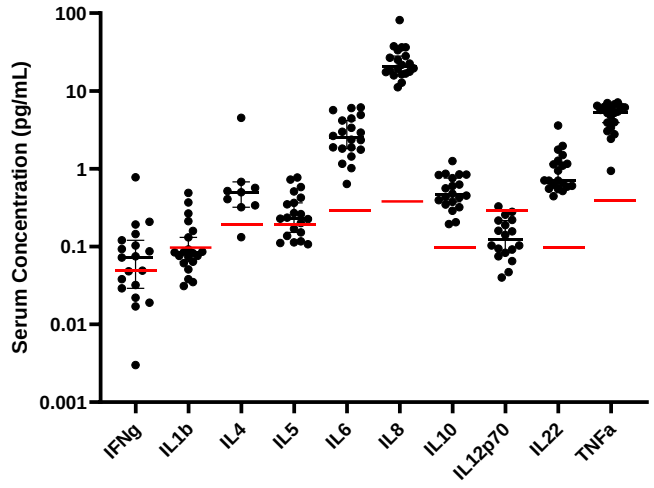
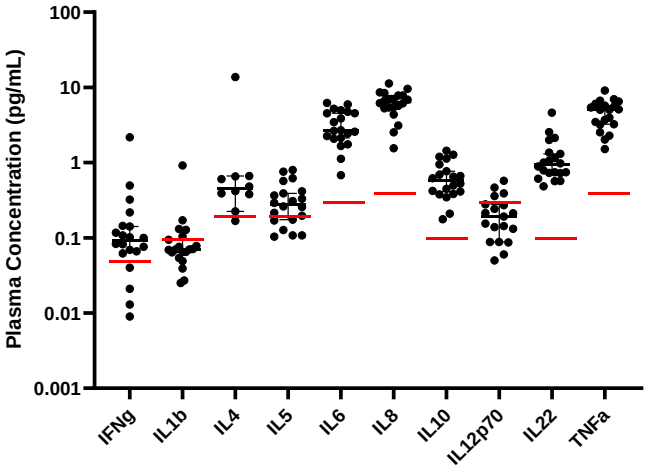
Endogenous Serum and Plasma Readings: Healthy donor matched EDTA plasma (n=20) and serum (n=20) samples were measured. Bars depict median with interquartile range. Red lines represent functional LLOQ.





	Sample Type	Median Conc pg/mL	% Above LOD	% Above LLOQ
IFNγ	Serum	0.07	75%	55%
	EDTA	0.09	85%	80%
IL-1β	Serum	0.08	85%	30%
	EDTA	0.07	85%	25%
IL-4	Serum	0.50	40%	30%
	EDTA	0.45	45%	30%
IL-5	Serum	0.23	100%	65%
	EDTA	0.28	100%	70%
IL-6	Serum	2.51	100%	100%
	EDTA	2.66	100%	100%
IL-8	Serum	20.62	100%	100%
	EDTA	6.39	100%	95%
IL-10	Serum	0.47	100%	100%
	EDTA	0.57	100%	100%
IL-12p70	Serum	0.12	45%	5%
	EDTA	0.19	70%	20%
IL-22	Serum	0.71	100%	100%
	EDTA	0.93	100%	100%
TNFα	Serum	5.33	100%	100%
	EDTA	5.04	100%	100%

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Precision: Measurements of 3 serum or plasma-based panels and 2 calibrator-based controls. Triplicate measurements were made across 3 reagent lots (13 runs total).

Mean (pg/mL)	IFNγ	IL-1β	IL-4	IL-5	IL-6	IL-8	IL-10	IL-12p70	IL-22	TNFα
Control 1	7.62	15.1	30.9	31.2	47.6	62.6	15.1	46.4	16.4	63.9
Control 2	0.35	0.74	1.74	1.56	2.35	2.79	0.68	4.71	0.78	2.76
Panel 1	15.2	0.59	41.8	72.2	72.2	352.0	0.73	113.5	28.1	125.2
Panel 2	1.49	4.00	4.59	8.47	10.8	15.09	4.63	12.4	2.85	29.2
Panel 3	1.33	17.2	1.05	1.30	1.49	1.80	0.29	1.48	0.26	19.8

Inter-run CV	IFNγ	IL-1β	IL-4	IL-5	IL-6	IL-8	IL-10	IL-12p70	IL-22	TNFα
Control 1	2.3%	4.8%	6.8%	3.9%	4.2%	10.1%	3.1%	4.2%	4.5%	4.3%
Control 2	6.0%	6.4%	4.7%	3.4%	4.5%	9.0%	5.1%	3.6%	8.4%	6.9%
Panel 1	2.5%	3.5%	3.8%	3.1%	1.5%	5.8%	5.4%	3.2%	3.4%	2.5%
Panel 2	4.0%	4.6%	7.8%	4.3%	3.6%	9.9%	3.2%	3.4%	7.6%	4.7%
Panel 3	4.5%	2.5%	12.0%	4.1%	4.3%	10.2%	6.7%	6.6%	6.4%	5.5%

Intra-run CV	IFNγ	IL-1β	IL-4	IL-5	IL-6	IL-8	IL-10	IL-12p70	IL-22	TNFα
Control 1	6.1%	6.9%	6.8%	4.9%	6.2%	11.3%	4.9%	6.5%	4.4%	7.2%
Control 2	16.3%	7.8%	14.9%	8.7%	11.3%	13.9%	10.0%	3.3%	11.5%	10.1%
Panel 1	5.6%	10.1%	3.8%	5.9%	5.8%	5.9%	15.7%	6.1%	5.9%	7.4%
Panel 2	8.2%	7.0%	9.5%	5.1%	7.4%	10.6%	5.5%	7.2%	8.3%	8.0%
Panel 3	16.8%	4.8%	25.5%	6.4%	9.5%	19.3%	13.7%	9.9%	13.9%	24.9%

Inter-lot CV	IFNγ	IL-1β	IL-4	IL-5	IL-6	IL-8	IL-10	IL-12p70	IL-22	TNFα
Control 1	3.3%	2.6%	2.1%	1.9%	3.2%	4.9%	8.4%	3.4%	1.6%	4.6%
Control 2	5.0%	2.5%	1.9%	5.0%	4.8%	8.7%	5.1%	2.1%	6.4%	5.2%
Panel 1	4.6%	6.0%	4.3%	3.3%	4.7%	1.9%	7.2%	2.3%	4.7%	6.5%
Panel 2	10.3%	2.9%	5.8%	3.7%	3.0%	6.5%	6.6%	6.7%	11.2%	5.6%
Panel 3	15.3%	4.9%	10.7%	6.8%	6.3%	14.4%	5.3%	6.3%	6.5%	17.2%

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

IFNγ	**Mean 101%** range 86%-114%
IL-1β	**Mean 90%** range 76%-100%
IL-4	**Mean 91%** range 79%-103%
IL-5	**Mean 90%** range 85%-102%
IL-6	**Mean 85%** range 51%-103%
IL-8	**Mean 105%** range 95%-113%
IL-10	**Mean 102%** range 98%-111%
IL-12p70	**Mean 93%** range 86%-106%
IL-22	**Mean 91%** range 81%-99%
TNFα	**Mean 100%** range 94%-107%

Dilution Linearity: 11 spiked serum and plasma samples were diluted 2X serially from 4x (MRD) to 256x with Sample Diluent.

IFNγ	**Mean 130%** range 105%-238%
IL-1β	**Mean 108%** range 98%-123%
IL-4	**Mean 120%** range 109%-133%
IL-5	**Mean 102%** range 86%-119%
IL-6	**Mean 113%** range 98%-127%
IL-8	**Mean 97%** range 86%-108%
IL-10	**Mean 105%** range 100%-116%
IL-12p70	**Mean 110%** range 100%-121%
IL-22	**Mean 121%** range 107%-131%
TNFα	**Mean 107%** range 93%-136%

Admixture Linearity: A serum sample from a normal healthy individual spiked with recombinant antigens was mixed in various ratios with a different unspiked serum sample. The average percent recovery across the entire dilution series is displayed for each sample in the table below.

IFNγ	**Mean 97%** range 72%-108%
IL-1β	**Mean 97%** range 87%-101%
IL-4	**Mean 97%** range 88%-103%
IL-5	**Mean 102%** range 92%-108%
IL-6	**Mean 102%** range 98%-105%
IL-8	**Mean 103%** range 97%-110%
IL-10	**Mean 109%** range 100%-116%
IL-12p70	**Mean 93%** range 83%-100%
IL-22	**Mean 102%** range 97%-108%
TNFα	**Mean 104%** range 96%-112%

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Comparison to NIBSC Standards: Biological Reference Materials obtained from the National Institute for Biological Standards and Control (NIBSC) were measured over multiple dilutions to generate a "NIBSC to CorPlex Ratio" (unitless). To compare CorPlex concentration relative to the NIBSC standard, multiply the CorPlex concentration by the ratio provided.

NIBSC Ref #	Analyte	NIBSC to CorPlex Ratio
87/586	**IFNγ**	**1.11**
86/680	**IL-1β**	**1.72**
88/656	**IL-4**	**1.52**
89/548	**IL-6**	**1.17**
89/520	**IL-8**	**0.63**
93/722	**IL-10**	**1.07**
95/544	**IL-12p70**	**0.87**
12/154	**TNFα**	**1.40**

Freeze/Thaw Stability: Five normal unspiked samples and 3 normal spiked samples were subjected to 1, 2 or 3 freeze/thaw cycles. The average percent change observed for each cycle is shown in the table below.

	0 F/T	1 F/T	2 F/T	3 F/T
IFNγ	100%	131%	96%	119%
IL-1β	100%	105%	97%	98%
IL-4	100%	153%	112%	165%
IL-5	100%	111%	101%	108%
IL-6	100%	105%	100%	105%
IL-8	100%	105%	100%	105%
IL-10	100%	112%	103%	110%
IL-12p70	100%	99%	93%	100%
IL-22	100%	108%	96%	107%
TNFα	100%	111%	100%	108%

Single-plex Correlation: Sample concentrations derived from single-plex standard curves were compared to the same samples calculated from the 10-plex standard curve. The average correlation between multi-plex and single-plex assays over the entire dynamic range is shown in the table below.

	Correlation
IFNγ	99%
IL-1β	107%
IL-4	112%
IL-5	105%
IL-6	109%
IL-8	98%
IL-10	106%
IL-12p70	98%
IL-22	103%
TNFα	104%

Specificity - Unrelated Antigens: Non-target recombinant human antigens were tested in the CorPlex assay. These antigens included MIP-1a, VEGF, Eotaxin, VEGF-C, GM-CSF, IL-18, IL-7, G-CSF, IP-10, MCSF, ICAM-1, MDC, VCAM-1, MCP-1, VEGF-D, RANTES, VEGF-R1, IL-6R, I-309, IFNα and MIP-1β. Non-specific binding was less than 0.08% of specific binding for all unrelated antigens. Percent non-specific binding was defined as non-specific signal/specific signal * 100.

Cross-reactivity: During assay validation, cross-reactivity was assessed by testing single antigen in the presence of all detection antibodies, and single detection antibodies in the presence of all antigens in assay buffer. In addition, cross-reactivity of single detection antibodies was assessed in sample matrix. These data are available in the validation report for this assay (Document number VAL-0014).

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