Simoa® Planar Array Mouse Cytokine Panel 1

SP-X™ Data Sheet Item 85-0008

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The Simoa Planar Array Mouse Cytokine Panel 1 is a multiplex immunoassay designed for the Quanterix SP-X™ Imaging and Analysis system, which simultaneously measures eight important cytokines in mouse serum and plasma. The eight proteins measured in the assay include Interferon gamma (IFNγ), IL-1β, IL-2, IL-6, IL-10, IL-12p70, IL-17A and Tumor Necrosis Factor alpha (TNFα).

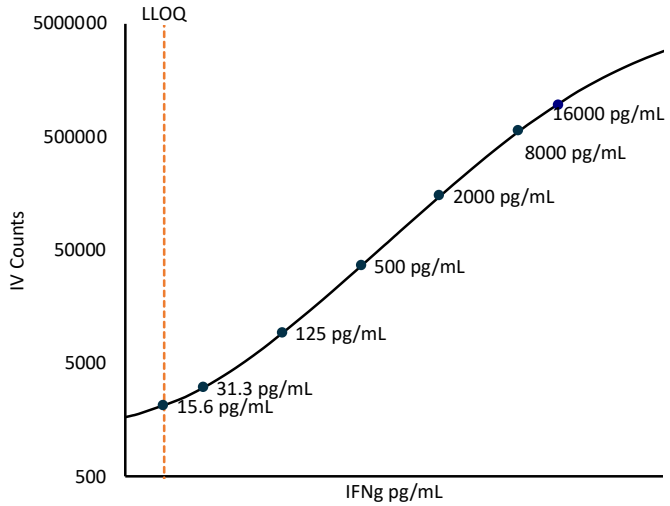
Description – Mouse IFNγ Test

Interferon-gamma (IFNγ), exerts a wide range of immunoregulatory activities Mature mouse IFN-gamma exists as a noncovalently linked homodimer of 20-25 kDa variably glycosylated subunits. IFNγ binds IFN-γ receptors (IFNγ R1 and IFNγ R2), which are expressed on most immune cells, to activate the JAK-STAT pathway. IFNγ-induced signaling increases the expression of class 1 major histocompatibility complex (MHC) molecules. IFNγ is produced by a variety of immune cells under inflammatory conditions, notably by T cells and NK cells. It plays a key role in host defense by promoting the development and activation of Th1 cells, chemoattraction and activation of monocytes and macrophages, and up-regulation of antigen presentation molecules. It also exhibits antiviral, antiproliferative, and apoptotic effects.

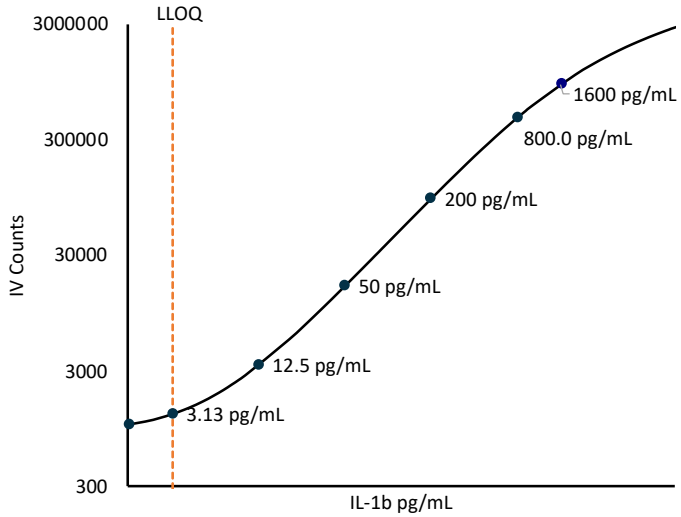
Description – Mouse IL-β Test

The IL-1 family consists primarily of three proteins: IL-1α, IL-1β (agonists) and IL-1ra (antagonist) which interact with the IL-1 receptor. IL-1β shares 33% homology with IL-1α. IL-1β exists as a 33 kDa precursor which is cleaved by caspase-1 into its 17 kDa active form. It is unknown how IL-1β is actively secreted but it is suggested exocytosis, transport by multi-drug resistance transporters, and cell death may all play a role. Knockout models of IL-1β show no gross physiological detriment, though its role is suspected to function in disease states rather than healthy tissue. Evidence shows potential involvement in Long Term Potentiation demonstrating increases following induction, and the prevention of induction with a competitive antagonist. IL-1β is believed to be part of an inflammatory response thought to be protective to insult and injury but often goes awry. There is a distinguishable link between oxidative stress, glutamate excitotoxicity and IL-1β.

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



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



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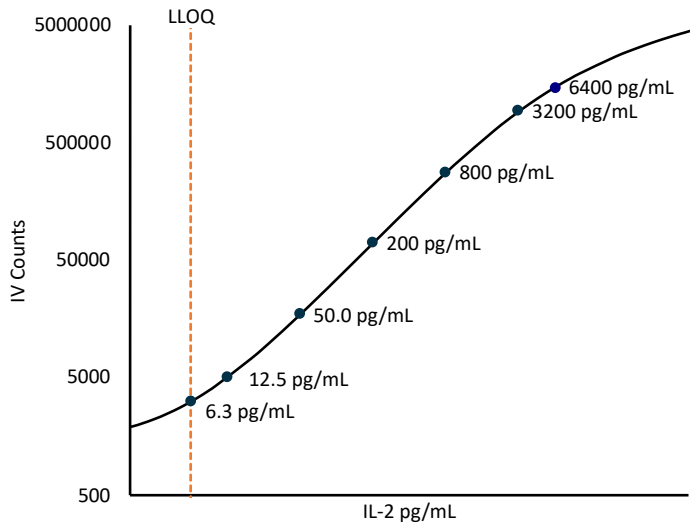
Description –Mouse IL-2 Test

Interleukin 2 (IL-2) is an alpha-helical cytokine of 153 amino acids (molecular weight 17.6kDa) whose primary role is regulation of activities of lymphocytes that are responsible for immunity. During infection, the binding of antigens to T cell receptors trigger secretion of IL-2 and expression of IL-2 receptors (IL-2R), promoting the growth, proliferation, and differentiation of T cells to become effector T cells. IL2/IL2R interaction stimulates growth and differentiation of antigen-specific CD4+ and CD8+ T cells, resulting in immunologic memory of the antigens. 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.

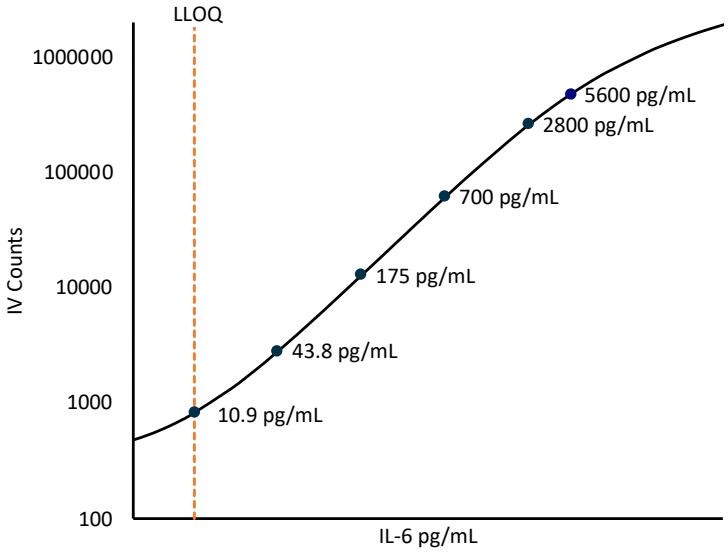
Mouse IL-2 Curve: Calibrator concentrations, and Lower Limit of Quantification depicted.



Description – Mouse 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. 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.

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



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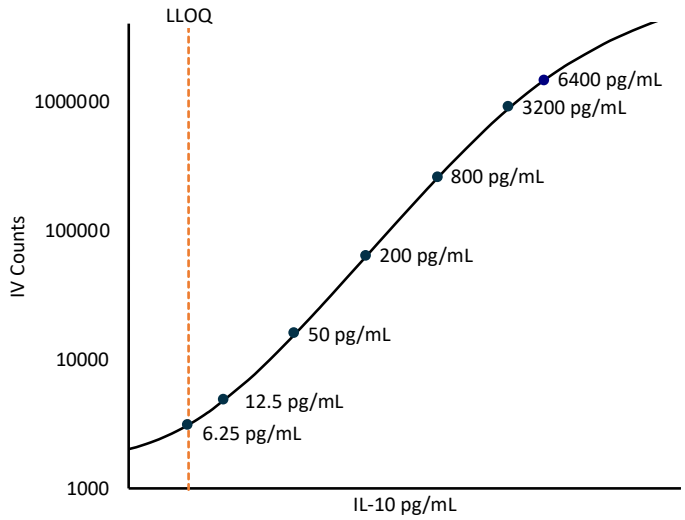
Description – Mouse IL-10 Test

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.

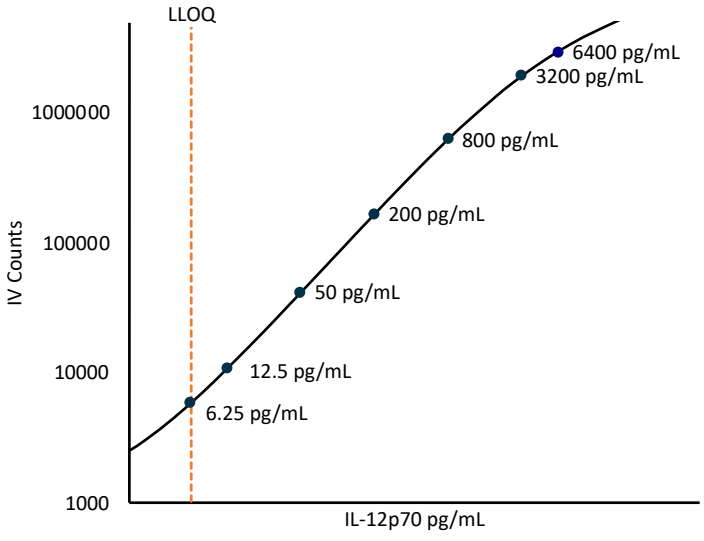
Mouse IL-10 Curve: Calibrator concentrations, and Lower Limit of Quantification depicted.



Description – Mouse 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 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.

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



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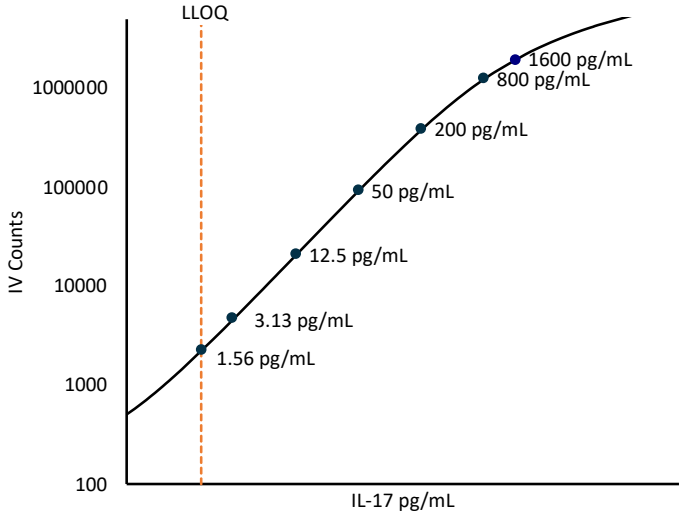
Description – Mouse IL-17A Test

Mouse Interleukin 17A (IL-17A) is a cytokine of 158 amino acids (molecular weight 21 kDa) and a member of an IL-17 family of related cytokines (IL-17B through IL-17F). IL-17 cytokines are well conserved in mammals, with significant sequence conservation between the human and mouse homologs. A major role of IL-17A is its involvement in inducing and mediating proinflammatory responses. It acts as potent mediator in delayed-type reactions by increasing chemokine production in various tissues to recruit monocytes and neutrophils to the site of inflammation, similar to interferon gamma. IL-17A is produced by T-helper cells and is induced by IL–23 which results in destructive tissue damage in delayed-type reactions. IL-17 induces the production of many other synergistic cytokines, including GM-CSF, IL-6, IL-1b, and TNFα. The IL-17 family has been linked to many immune/autoimmune related diseases including rheumatoid arthritis, asthma, lupus, allograft rejection, anti-tumor immunity and recently Psoriasis. Because of its involvement in autoimmune conditions, IL-17 inhibitors are being investigated as possible treatments.

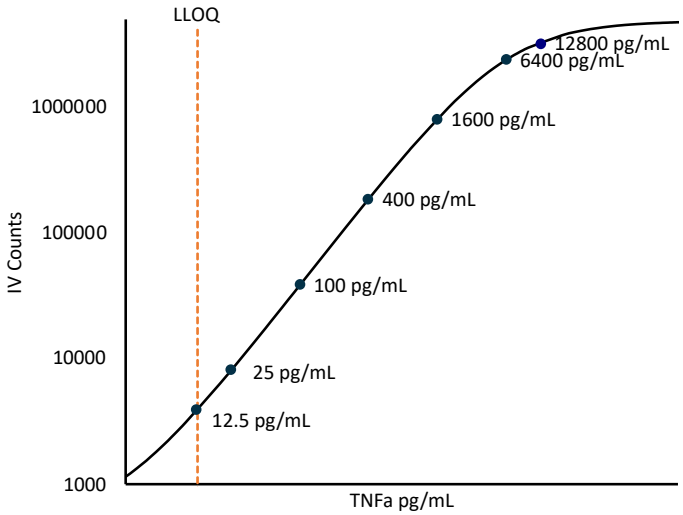
Description – Mouse TNFα Test

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.

Mouse IL-17A Curve: Calibrator concentrations, and Lower Limit of Quantification depicted.



Mouse TNFα 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 (7 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)
mIFNγ	**15.63 pg/mL**	**31.25 pg/mL**
mIL-1β	**3.12 pg/mL**	**6.25 pg/mL**
mIL-2	**6.25 pg/mL**	**12.5 pg/mL**
mIL-6	**10.94 pg/mL**	**21.88 pg/mL**
mIL-10	**6.25 pg/mL**	**12.5 pg/mL**
mIL-12p70	**6.25 pg/mL**	**12.5 pg/mL**
mIL-17	**1.56 pg/mL**	**3.12 pg/mL**
mTNFα	**12.5 pg/mL**	**24.4 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 (7 runs total).

	LOD
mIFNγ	**8.62 pg/mL** Range 3.60 – 14.33
mIL-1β	**1.87 pg/mL** Range 0.22 – 2.84
mIL-2	**1.66 pg/mL** Range 0.25 – 3.75
mIL-6	**3.44 pg/mL** Range 0.87 – 5.88
mIL-10	**4.98 pg/mL** Range 0.2.90 – 7.80
mIL-12p70	**1.47 pg/mL** Range 0.32 – 2.24
mIL-17	**0.38 pg/mL** Range 0.22 – 0.49
mTNFα	**0.63 pg/mL** Range 0.18 – 2.26

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

	Assay Range
mIFNγ	0 – 16,000 pg/mL
mIL-1β	0 – 1,600 pg/mL
mIL-2	0 – 6,400 pg/mL
mIL-6	0 – 5,600 pg/mL
mIL-10	0 – 6,400 pg/mL
mIL-12p70	0 – 6,400 pg/mL
mIL-17	0 – 1,600 pg/mL
mTNFα	0 – 12,800 pg/mL

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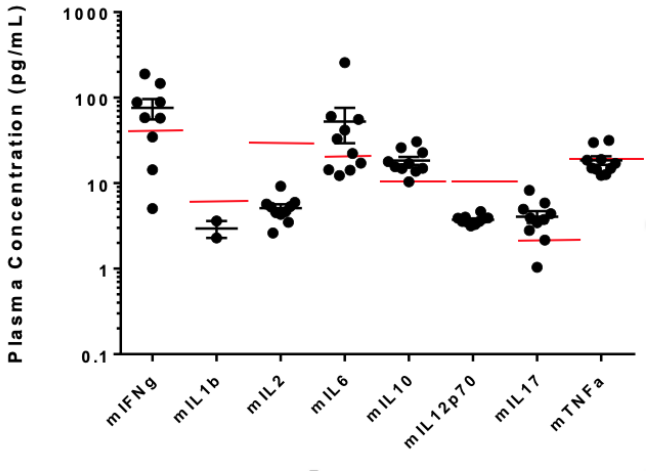
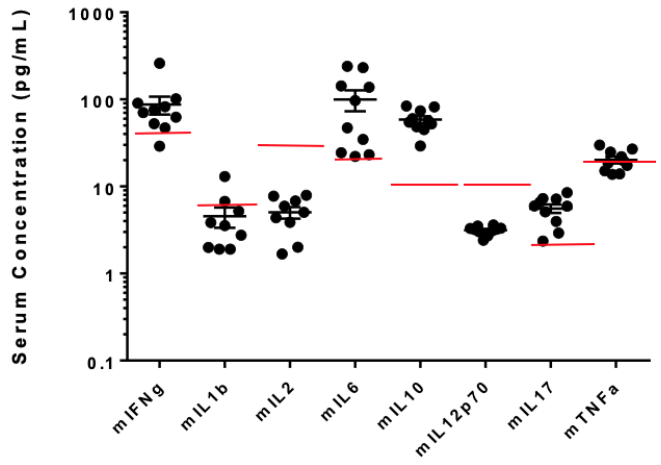
Endogenous Serum and Plasma Readings EDTA plasma (n=10) and serum (n=10) samples from CD-1 (ICR) mice were measured. Bars depict median with interquartile range. Red lines represent functional LLOQ.





	Sample Type	Median Conc pg/mL	% Above LOD	% Above LLOQ
mIFNγ	Serum	87.26	100%	90%
	EDTA	75.94	90%	70%
mIL-1β	Serum	4.54	90%	10%
	EDTA	2.95	20%	0%
mIL-2	Serum	5.04	90%	10%
	EDTA	5.10	100%	0%
mIL-6	Serum	99.94	100%	100%
	EDTA	52.67	100%	60%
mIL-10	Serum	58.66	100%	100%
	EDTA	18.36	100%	90%
mIL-12p70	Serum	3.14	100%	0%
	EDTA	3.75	100%	0%
mIL-17	Serum	5.58	100%	80%
	EDTA	4.06	100%	70%
mTNFα	Serum	20.17	100%	20%
	EDTA	18.54	100%	20%

Dilution Linearity: Two spiked serum and 1 spiked plasma samples were diluted 2x according to the MRD, and then serially diluted 2x with Sample Diluent eight times, for final dilution of 1:512.

mIFNγ	**Mean 81.6 %** Range 74.0%–91.6 %
mIL-1β	**Mean 107.5%** Range 96.7%–140.0%
mIL-2	**Mean 54.6%** Range 47.5%–63.6%
mIL-6	**Mean 107.2%** Range 90.9%–132.6%
mIL-10	**Mean 50.7%** Range 44.2%–61.6%
mIL-12p70	**Mean 155.4%** Range 110.8%–209.5%
mIL-17	**Mean 139.3%** Range 107.3%–193.1 %
mTNFα	**Mean 102.1 %** Range 92.0%–128.2%

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

mIFNγ	**Mean 119%** Range 79%–141%
mIL-1β	**Mean 112%** Range 106%–118%
mIL-2	**Mean 120%** Range 75%–183%
mIL-6	**Mean 161%** Range 127%–183%
mIL-10	**Mean 175%** Range 123%–246%
mIL-12p70	**Mean 98%** Range 75%–129%
mIL-17	**Mean 89%** Range 67%–116%
mTNFα	**Mean 132%** Range 94%–169%

Single-plex Correlation: Sample concentrations derived from single-plex standard curves were compared to the same samples calculated from the 8-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
mIFNγ	1.2%
mIL-1β	–15.6%
mIL-2	–5.0%
mIL-6	–11.7%
mIL-10	14.3%
mIL-12p70	8.7%
mIL-17	8.4%
mTNFα	0.5%

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Precision: Measurements of 3 diluent-based controls. Triplicate measurements were made across a single reagent lot (7 runs total).

Mean (pg/mL)	mIFNγ	mIL-1β	mIL-2	mIL-6	mIL-10	mIL-12p70	mIL-17	mTNFα
Control H	2,929.9	563.6	2,208.8	1,792.4	2,047.3	2,269.7	566.1	4,165.6
Control M	413.2	99.3	366.7	348.8	406.5	405.0	105.3	756.9
Control L	76.8	21.9	79.2	63.3	78.9	90.4	21.2	148.8

Inter-run CV	mIFNγ	mIL-1β	mIL-2	mIL-6	mIL-10	mIL-12p70	mIL-17	mTNFα
Control H	9.4%	6.4%	11.2%	13.0%	10.7%	14.2%	4.7%	14.1%
Control M	10.8%	9.1%	8.6%	12.3%	9.2%	7.8%	5.1%	16.0%
Control L	17.6%	10.1%	11.3%	7.0%	12.6%	13.1%	7.9%	18.3%

Intra-run CV	mIFNγ	mIL-1β	mIL-2	mIL-6	mIL-10	mIL-12p70	mIL-17	mTNFα
Control H	2.7%	2.9%	5.5%	5.5%	2.8%	6.2%	5.3%	5.0%
Control M	5.0%	4.6%	9.3%	5.7%	7.2%	10.0%	4.3%	2.5%
Control L	14.0%	9.9%	8.1%	10.3%	8.2%	12.2%	5.2%	4.3%

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 3.8% of cross reactivity), and single detection antibodies in the presence of all antigens (maximum 3.7% of cross reactivity) in assay buffer. In addition, no cross-reactivity of single detection antibodies was observed in sample matrix.

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