Simoa® Cytokine 6-plex Panel 1 Kit
SR-X® Data Sheet

Item 102958

Human Cytokine 6-plex panel 1 assay (C6P1)

The Simoa Human Cytokine 6-plex Panel 1 assay (C6P1) simultaneously measures six cytokines in serum and EDTA plasma. The targets are Interferon gamma (IFNγ), IL-10, IL-12p70, IL-17A, IL-6, and Tumor Necrosis Factor alpha (TNFα).

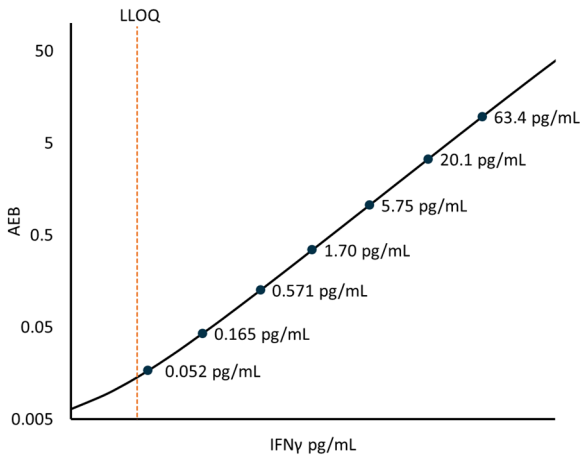
Description – IFNγ Test

Human interferon-gamma (IFN-γ) is a dimeric cytokine with subunits of 146 amino acids. Mature human IFN-γ exists as a non-covalently linked homodimer of 20-25 kDa variably glycosylated subunits. IFN-γ does not display significant homology with the other two interferons, IFN-α and IFN-β. Murine and human IFN-γ show approximately 40% sequence homology at the protein level. IFN-γ is expressed by Th1 cells, Tc cells, dendritic cells and natural killer cells, especially under inflammatory conditions. IFN-γ binds to its heterodimeric receptor IFN-γR and related complex for biological function. It plays a key role in host defense by promoting the development and activation of Th1 cells, chemoattraction and activation of monocytes and macrophages, upregulation of antigen presentation molecules, and immunoglobulin class switching in B cells. In addition, IFN-γ functions as an anti-inflammatory mediator by promoting the development of regulatory T cells and inhibiting Th17 cell differentiation. It also exhibits antiviral, antiproliferative, and apoptotic effects. IFN-γ is an attractive drug target for immuno-regulatory diseases

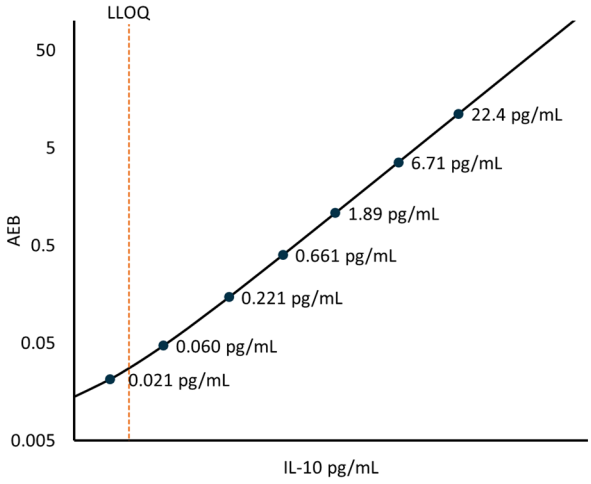
Description – IL-10 Test

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.

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



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

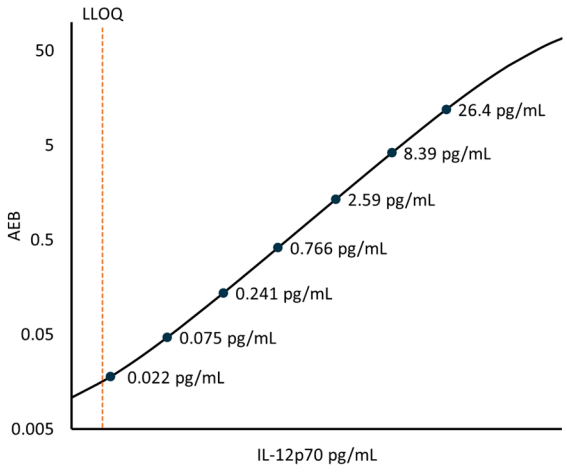


Quanterix Corporation
900 Middlesex Turnpike, Billerica, MA 01821
techsupport@quanterix.com

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

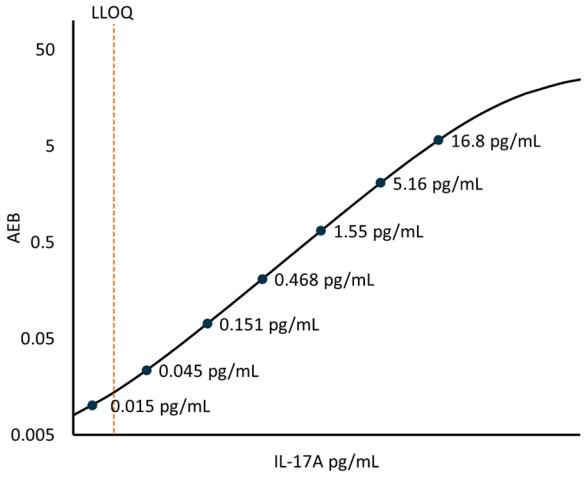
IL-12p70 Curve: Calibrator concentrations and Lower Limit of Quantification depicted.



Description – IL-17A Test

Interleukin 17A (IL-17A) is disulfide-linked homodimeric cytokine of 155 amino acids (molecular weight 35kDa) and a member of an IL-17 family of related cytokines (IL-17B through IL-17F). All IL-17 cytokines have a similar protein structure, and no sequence similarity to any other cytokines. These 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 a 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 TNFa. 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.

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



Quanterix Corporation
900 Middlesex Turnpike, Billerica, MA 01821
techsupport@quanterix.com

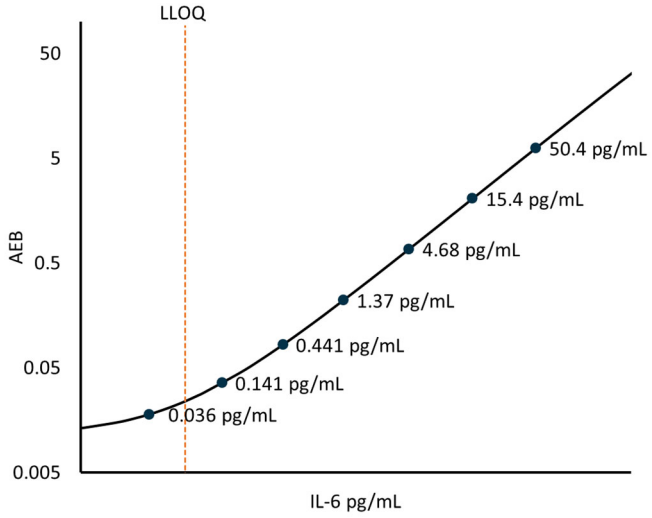
Description – IL-6 Test

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.

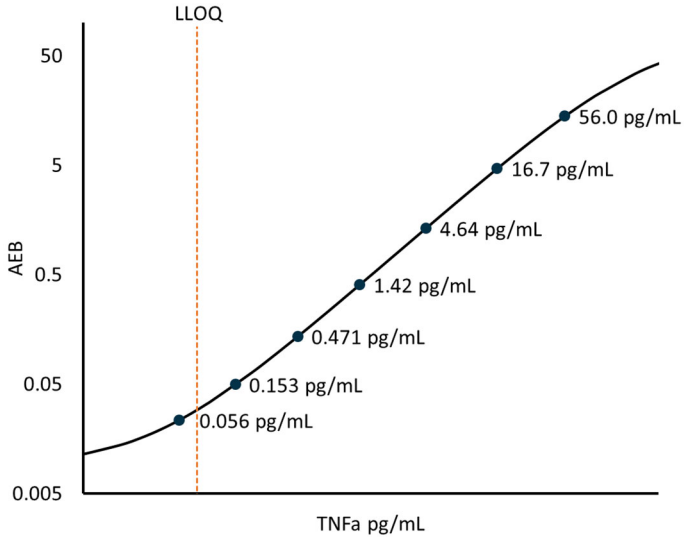
Description – TNFα Test

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



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



Quanterix Corporation
900 Middlesex Turnpike, Billerica, MA 01821
techsupport@quanterix.com

Minimum Required Dilution (MRD)

Diluted Sample Volume	100 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 lot across 2 instruments (12 runs total). The functional LLOQ (fLLOQ) values below are for serum and plasma.

	Analytical LLOQ	Functional LLOQ (x MRD)
IFNγ	**0.041 pg/mL** pooled CV **19.2**% mean recovery **101**%	**0.082 pg/mL**
IL-10	**0.030 pg/mL** pooled CV 18.5% mean recovery 110%	**0.060 pg/mL**
IL-12p70	**0.019 pg/mL** pooled CV 18.0% mean recovery 92.7%	**0.038 pg/mL**
IL-17A	**0.023 pg/mL** pooled CV 19.8% mean recovery 103%	**0.046 pg/mL**
IL-6	**0.071 pg/mL** pooled CV 17.4% mean recovery 95.1%	**0.142 pg/mL**
TNFα	**0.077 pg/mL** pooled CV 15.9% mean recovery 110%	**0.154 pg/mL**

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 2 reagent lot across 2 instruments (12 runs total).

	LOD
IFNγ	**0.010 pg/mL** range 0.002-0.016 pg/mL
IL-10	**0.006 pg/mL** range 0.001-0.014 pg/mL
IL-12p70	**0.006 pg/mL** range 0.003-0.008 pg/mL
IL-17A	**0.006 pg/mL** range 0.001-0.012 pg/mL
IL-6	**0.021 pg/mL** range 0.009-0.035 pg/mL
TNFα	**0.018 pg/mL** range 0.006-0.032 pg/mL

Assay Range: The upper end of the dynamic range is equal to the top calibrator concentration multiplied by MRD. The ranges below are for serum and plasma.

	Assay Range
IFNγ	0 - 140 pg/mL
IL-10	0 - 50 pg/mL
IL-12p70	0 - 50 pg/mL
IL-17A	0 - 20 pg/mL
IL-6	0 - 140 pg/mL
TNFα	0 - 100 pg/mL

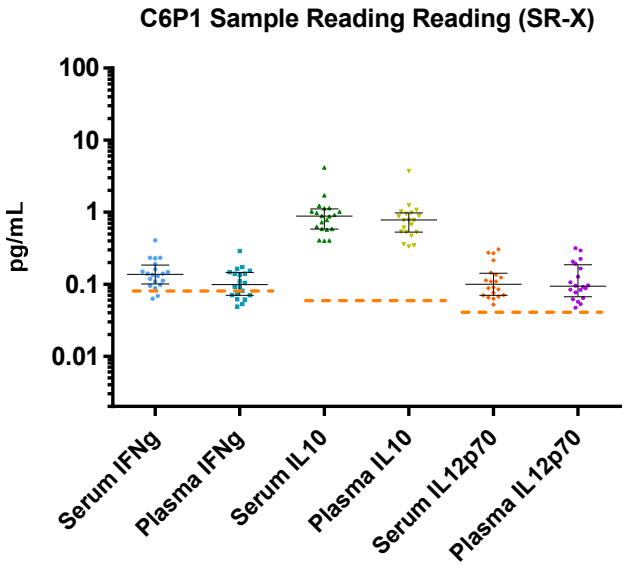
Endogenous Sample Reading: Healthy donor matched EDTA plasma (n=20), and serum (n=20) were measured. Bars depict median with interquartile range. Orange line represents functional LLOQ. IFNγ and IL-17a contain values below LLOQ that are not included in the mean calculation.

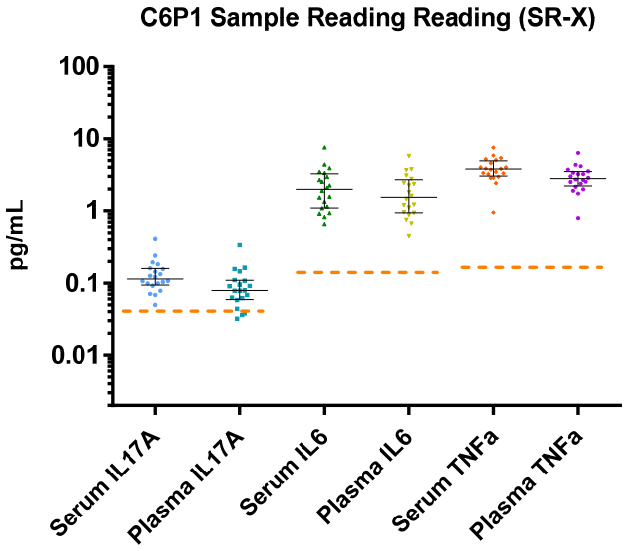
Quanterix Corporation
900 Middlesex Turnpike, Billerica, MA 01821
techsupport@quanterix.com

Simoa® Cytokine 6-plex Panel 1 Kit

SR-X® Data Sheet

Item 102958





	Sample Type	Mean Conc pg/mL	Median Conc pg/mL	% Above LOD	% Above LLOQ
IFNγ	Plasma	0.145	0.098	100%	65.0%
	Serum	0.162	0.137	100%	85.0%
IL-10	Plasma	0.875	0.777	100%	100%
	Serum	1.005	0.882	100%	100%
IL-12p70	Plasma	0.126	0.094	100%	100%
	Serum	0.126	0.099	100%	100%

	Sample Type	Mean Conc pg/mL	Median Conc pg/mL	% Above LOD	% Above LLOQ
IL-17A	Plasma	0.111	0.078	100%	90%
	Serum	0.137	0.115	100%	100%
IL-6	Plasma	1.961	1.540	100%	100%
	Serum	2.357	1.995	100%	100%
TNFα	Plasma	2.968	2.799	100%	100%
	Serum	3.934	3.789	100%	100%

Precision: Measurements of 3 serum- or plasma-based panels and 2 calibrator-based controls. Triplicate measurements were made for 6 runs each for 2 reagent lot across 2 instruments (12 runs total, 36 measurements).

IFNγ	Mean (pg/mL)	Within run CV	Between run CV	Between inst CV	Between Lot CV
Control 1	1.69	6.8%	9.8%	1.7%	0.5%
Control 2	42.3	8.2%	6.0%	2.0%	0.6%
Panel 1	0.223	15.2%	12.9%	2.9%	0.7%
Panel 2	18.9	8.5%	13.1%	0.6%	3.1%
Panel 3	60.0	8.6%	12.0%	11.8%	0.8%

IL-10	Mean (pg/mL)	Within run CV	Between run CV	Between inst CV	Between Lot CV
Control 1	0.907	3.8%	3.7%	0.3%	2.6%
Control 2	18.3	5.4%	4.9%	0.9%	6.0%
Panel 1	0.902	5.5%	5.2%	2.9%	2.1%
Panel 2	8.92	7.3%	5.5%	3.9%	3.2%
Panel 3	27.0	6.3%	10.3%	8.1%	1.8%

IL-12p70	Mean (pg/mL)	Within run CV	Between run CV	Between inst CV	Between Lot CV
Control 1	1.02	3.4%	4.5%	3.6%	1.5%
Control 2	20.6	4.8%	3.6%	1.0%	1.9%
Panel 1	0.226	6.7%	3.6%	1.9%	1.7%
Panel 2	6.33	6.5%	7.9%	2.3%	4.4%
Panel 3	23.8	5.3%	8.9%	6.7%	1.5%

Quanterix Corporation
900 Middlesex Turnpike, Billerica, MA 01821
techsupport@quanterix.com

IL-17A	Mean (pg/mL)	Within run CV	Between run CV	Between inst CV	Between Lot CV
Control 1	0.413	6.6%	6.3%	3.0%	1.5%
Control 2	12.4	6.1%	4.1%	2.1%	1.3%
Panel 1	0.260	8.4%	5.6%	4.1%	3.6%
Panel 2	7.89	8.0%	9.7%	2.5%	2.0%
Panel 3	24.5	7.2%	9.0%	9.2%	1.7%

IL-6	Mean (pg/mL)	Within run CV	Between run CV	Between inst CV	Between Lot CV
Control 1	1.36	4.8%	3.7%	3.4%	5.8%
Control 2	32.8	4.7%	4.1%	0.8%	4.9%
Panel 1	4.63	5.2%	4.2%	3.3%	2.4%
Panel 2	12.24	5.7%	4.8%	1.5%	4.8%
Panel 3	41.1	6.0%	10.2%	7.8%	9.1%

TNFα	Mean (pg/mL)	Within run CV	Between run CV	Between inst CV	Between Lot CV
Control 1	1.06	7.2%	8.9%	2.3%	4.1%
Control 2	40.7	6.2%	4.6%	0.5%	0.6%
Panel 1	3.08	7.1%	5.4%	2.2%	1.6%
Panel 2	11.10	8.2%	7.2%	1.9%	2.9%
Panel 3	47.2	6.7%	8.8%	8.1%	2.0%

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 SR-X.

	Recovery
IFNγ	**86.7%** range 59.7-133%
IL-10	**94.3%** range 87.7-103%
IL-12p70	**74.4%** range 55.3-93.5%
IL-17A	**95.2%** range 70.3-122%
IL-6	**83.5%** range 70.8-106%
TNFα	**96.0%** range 81.6-114%

Dilution Linearity: 1-4 endogenous or spiked EDTA plasma endogenous serum samples were diluted 2x serially from MRD (2x) to 256x with Sample Diluent.

Linearity				
	Matrix	**Source**	**Dilution Factor**	**Results**
IFNγ	Serum	Spiked	32	82%
	Serum	Endogenous	8	96%
	Plasma	Spiked	128	95%
	Plasma	Endogenous	8	94%
IL-10	Serum	Endogenous	16	97%
	Plasma	Endogenous	16	102%
IL-12p70	Serum	Endogenous	8	106%
	Plasma	Endogenous	8	119%
	Plasma	Spiked	256	93%
IL-17A	Serum	Spiked	128	85%
	Serum	Endogenous	8	110%
	Plasma	Spiked	256	95%
	Plasma	Endogenous	8	114%
IL-6	Serum	Endogenous	16	96%
	Plasma	Endogenous	8	98%
TNFα	Serum	Endogenous	32	98%
	Plasma	Endogenous	32	95%

The Simoa Cytokine 6-plex Panel 1 assay kit is formulated for use on the SR-X and HD-X platform. Some differences in performance claims between the HD-X and SR-X platforms may be observed when comparing data sheets for these 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.

Quanterix Corporation
900 Middlesex Turnpike, Billerica, MA 01821
techsupport@quanterix.com