Simoa® Cytokine 4-Plex C (C4PC) Advantage PLUS Kit
HD-X Data Sheet

Item 105066

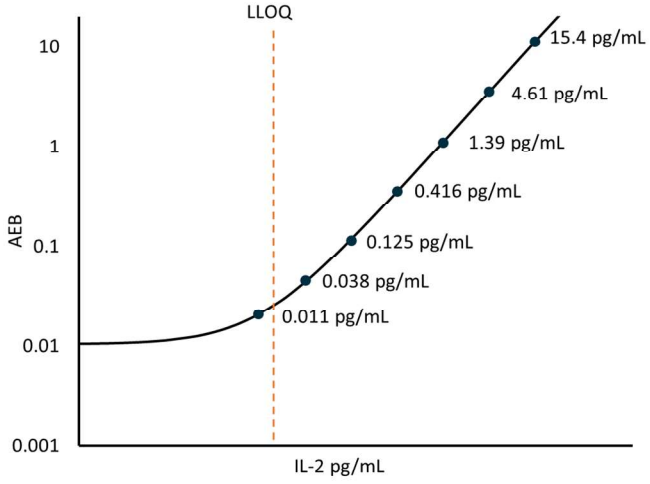
Description

This datasheet summarizes data from analytical validation performed at Quanterix to characterize performance of the Cytokine 4-Plex C (C4PC) kit on the HD-X platform. Data provided includes Calibration Curves, Minimum Required Dilution (MRD), Lower Limit of Qualification (LLOQ), Limit of Detection (LOD), Assay Range, Precision, Spike and Recovery, and Dilution Linearity.

IL-2

Interleukin 2 (IL-2) is a four alpha helix bundle with a glycosylated 15.5-16 kDa cytokine. Human and murine IL-2 and IL-2 receptor (R) subunits have 60-70% homology. IL-2 has a part of the body natural response to microbial infection and mediates interactions between leukocytes. IL-2 binds to IL-2R alpha subunit with low affinity and hetero-dimerization of the beta (β) and gamma (γ) subunits of IL-2R increases the affinity. the β and γ subunits is crucial for the signaling in T cells. IL-2 is activated $CD4^+$ and $CD8^+$ T cells and stimulate the helper T cells, cytotoxic and regulatory T cells. IL-2 up-regulation plays a central role in enduring cell-mediated immunity. This cytokine induces cell proliferation of natural killer cells; cells of the innate immune system whose role is to kill virally infected cells. As a result of this ability, IL-2 may have a role as a potential immunotherapy for cancer and autoimmune diseases.

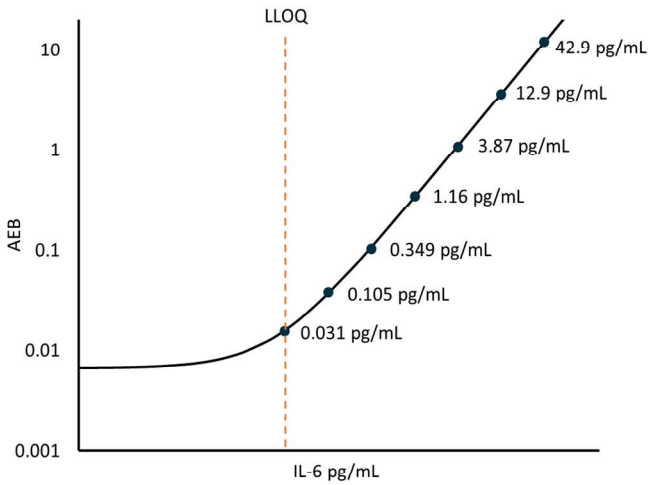
IL-2 Curve: Representative calibrator concentrations and Lower Limit of Quantification (LLOQ) depicted.



IL-6

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 22-28 kDa phosphorylated and variably glycosylated molecule. Mature human IL-6 is 183 amino acids (aa) in length and shows 41% sequence homology 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 autoimmune response, there is interest in developing anti-IL-6 agents as potential therapies against various diseases, including rheumatoid arthritis and cancer.

IL-6 Curve: Representative calibrator concentrations and Lower Limit of Quantification (LLOQ) depicted.



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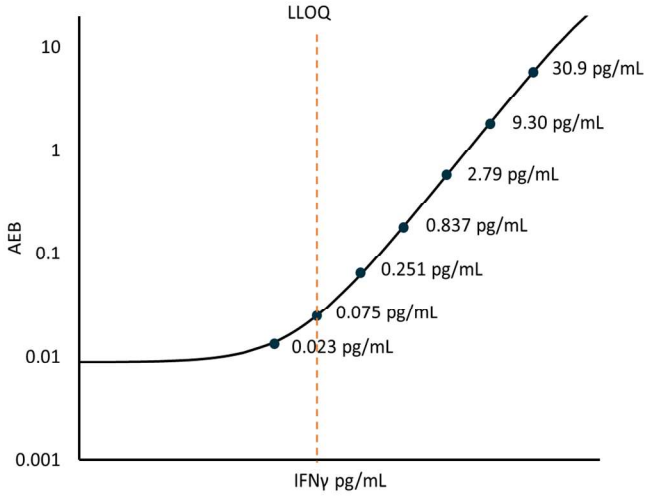
IFNγ

Human interferon-gamma (IFNγ) is a dimeric cytokine with subunits of 146 amino acids. Mature human IFNγ exists as a non-covalently linked homodimer of 20-25 kDa variably glycosylated subunits. IFNγ does not display significant homology with the other two interferons, IFNα and 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. IFNγ binds to its heterodimeric receptor IFNγR 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 monocyte and macrophages, upregulation of antigen presentation molecules, an immunoglobulin class switching in B cells. In addition, IFNγ 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. IFNγ is an attractive drug target for immunoregulatory diseases.

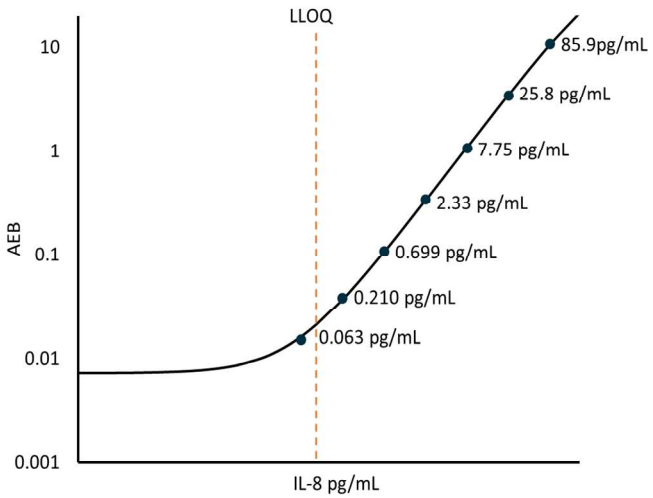
IL-8

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 oxidative stress and results in the recruitment of inflammatory cells. This recruitment further induces oxidative stress mediators, making it a key player in localized inflammation. IL-8 elevation has been associated with a range of clinical conditions, including psoriasis, chronic hepatitis C, ant thyroid disease. IL-8 has recently been identified as a potential therapeutic target in inflammatory diseases.

IFNγ Curve: Representative calibrator concentrations and Lower Limit of Quantification (LLOQ) depicted.



IL-8 Curve: Representative calibrator concentrations and Lower Limit of Quantification (LLOQ) depicted.



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Minimum Required Dilution (MRD)

Diluted Sample Volume	100 µL per measurement
Serum/EDTA Plasma Dilution	1:4
Tests per Kit	96

See Kit Instruction for details.

Lower Limit of Quantification (LLOQ): The analytical LLOQ was set at the lowest concentration that read back within 80 – 120% of the expected value with a CV ≤ 20%. The functional LLOQ (fLLOQ) values below are for serum and EDTA plasma and represent the analytical LLOQ multiplied by the dilution factor used for the samples.

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 (3 runs per lot, per instrument, 12 runs total).

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

IL-2	
Analytical LLOQ	0.019 pg/mL Pooled CV: 13% Recovery: 102%
Functional LLOQ	Serum/EDTA Plasma (4x): 0.076 pg/mL
LOD	0.005 pg/mL Range: 0.001 - 0.009 pg/mL
Dynamic Range	Serum/EDTA Plasma (4x): 0 - 80 pg/mL

IL-6	
Analytical LLOQ	0.031 pg/mL Pooled CV: 18% Recovery: 103%
Functional LLOQ	Serum/EDTA Plasma (4x): 0.124 pg/mL
LOD	0.008 pg/mL Range: 0.003 - 0.017 pg/mL
Dynamic Range	Serum/EDTA Plasma (4x): 0 - 160 pg/mL

IFNγ	
Analytical LLOQ	0.075 pg/mL Pooled CV: 8% Recovery: 100%
Functional LLOQ	Serum/EDTA Plasma (4x): 0.3 pg/mL
LOD	0.012 pg/mL Range: 0.003 - 0.022 pg/mL
Dynamic Range	Serum/EDTA Plasma (4x): 0 - 160 pg/mL

IL-8	
Analytical LLOQ	0.07 pg/mL Pooled CV: 19% Recovery: 112%
Functional LLOQ	Serum/EDTA Plasma (4x): 0.28 pg/mL
LOD	0.014 pg/mL Range: 0.006 - 0.03 pg/mL
Dynamic Range	Serum/EDTA Plasma (4x): 0 - 320 pg/mL

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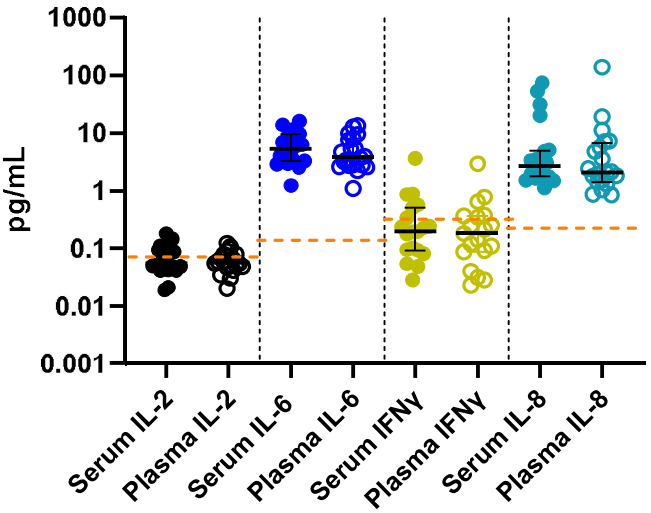
Endogenous Sample Reading: Concentrations (pg/mL) were determined for matched EDTA plasma (*n*=20), and serum (*n*=20) from normal human donors using the C4PC Advantage PLUS kit on HD-X. Bars depict median with interquartile range. Orange line represents functional LLOQ.



IL-2				
Sample	Mean (pg/mL)	Median (pg/mL)	% Above LOD	% Above LLOQ
Serum	0.115	0.108	95%	40%
EDTA Plasma	0.096	0.095	100%	30%

IL-6				
Sample	Mean (pg/mL)	Median (pg/mL)	% Above LOD	% Above LLOQ
Serum	6.6	5.42	100%	100%
EDTA Plasma	5.44	3.86	100%	100%

IFNγ				
Sample	Mean (pg/mL)	Median (pg/mL)	% Above LOD	% Above LLOQ
Serum	1.14	0.718	90%	35%
EDTA Plasma	0.914	0.514	80%	35%

IL-8				
Sample	Mean (pg/mL)	Median (pg/mL)	% Above LOD	% Above LLOQ
Serum	11.2	2.67	100%	100%
EDTA Plasma	11	2.11	100%	100%

*Values below LLOQ are excluded from the mean and median calculation.

Precision: Measurements of 2 calibrator-based controls, 3 commercial pooled serum panels and 3 commercial pooled plasma panels. Triplicate measurements were made for 6 runs each for 2 reagent lot across 2 instruments (12 runs total, 36 measurements). All samples were diluted at the appropriate MRD for the sample matrix.

IL-2					
Sample	Mean (pg/mL)	Within Run CV	Between Run CV	Between Lot CV	Between Instr CV
Control 1	0.331	5.1%	8.5%	4.3%	2.8%
Control 2	9.02	3.2%	10%	5.3%	4.9%
Panel 1	7.42	3.0%	9.9%	6.0%	2.7%
Panel 2	17.6	2.7%	8.9%	4.4%	3.4%
Panel 3	0.489	4.2%	7.5%	3.2%	0.3%
Panel 4	7.98	3.2%	8.9%	5.7%	2.1%
Panel 5	17.4	5.7%	10%	4.2%	3.9%
Panel 6	0.632	3.9%	8.9%	2.4%	2.7%

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IL-6					
Sample	Mean (pg/mL)	Within Run CV	Between Run CV	Between Lot CV	Between Instr CV
Control 1	0.930	6.5%	9.7%	2.9%	3.3%
Control 2	24.9	3.7%	8.5%	3.3%	3.4%
Panel 1	41.3	3.2%	9.2%	5.4%	1.2%
Panel 2	36.0	3.5%	8.4%	3.8%	1.9%
Panel 3	2.93	4.2%	10%	5.8%	1.1%
Panel 4	39.5	3.2%	8.5%	5.2%	0.6%
Panel 5	33.0	5.5%	8.6%	3.6%	2.4%
Panel 6	3.19	3.4%	9.2%	3.1%	0.5%

IFNγ					
Sample	Mean (pg/mL)	Within Run CV	Between Run CV	Between Lot CV	Between Instr CV
Control 1	0.701	6.3%	8.7%	2.5%	8.8%
Control 2	18.6	3.3%	11%	4.5%	12%
Panel 1	23.9	3.2%	11%	2.6%	11%
Panel 2	20.1	2.8%	10%	4.3%	8.1%
Panel 3	0.901	5.4%	9.1%	1.5%	3.8%
Panel 4	24.3	4.6%	10%	1.8%	7.3%
Panel 5	20.6	5.9%	10%	4.4%	6.3%
Panel 6	1.93	5.2%	10%	2.5%	10%

IL-8					
Sample	Mean (pg/mL)	Within Run CV	Between Run CV	Between Lot CV	Between Instr CV
Control 1	1.89	7.6%	10%	4.8%	1.2%
Control 2	49.7	4.1%	11%	1.2%	4.5%
Panel 1	57.5	3.7%	9.4%	1.9%	1.4%
Panel 2	48.7	3.0%	8.1%	0.5%	1.0%
Panel 3	0.977	7.9%	13%	4.4%	3.6%
Panel 4	52.0	3.9%	8.2%	3.1%	1.3%
Panel 5	42.5	6.2%	9.1%	1.0%	1.1%
Panel 6	3.71	4.5%	12%	1.5%	0.8%

Spike and Recovery: 2 serum and 2 EDTA plasma samples were spiked at high and low concentrations of IL-2, IL-6, IFNγ, and IL-8 within the range of each assay and analyzed on HD-X. Percent recovery is defined as the difference between the measured concentration of the analytes in the spiked sample and the measured concentration in unspiked sample relative to the concentration of the analytes in spiked calibrator diluent. Results indicate that Matrix effects are observed with the IL-2, IL-6, and IL-8 assays, as a limited dilution was chosen to maximize the detectability / quantifiability of the analyte in samples from healthy donors.

Dilution Linearity: 2 serum and 2 EDTA plasma samples were spiked with endogenous antigen and serially diluted 2X with sample diluent and then tested at 2XMRD. Total dilution of each sample ranged from 4x to 64x. For valid comparison between results, it is recommended to run all samples at a consistent dilution.

IL-2	
Spike and Recovery Serum	Mean: 82.1% Range: 70.6 - 93%
Spike and Recovery EDTA Plasma	Mean: 65.1% Range: 58.4 - 70.2%
Dilution Linearity Serum	Mean: 108% Range: 99.4 - 134%
Dilution Linearity EDTA Plasma	Mean: 108% Range: 97.7 - 119%

IL-6	
Spike and Recovery Serum	Mean: 92.7% Range: 85 - 106%
Spike and Recovery EDTA Plasma	Mean: 78.1% Range: 71.8 - 87%
Dilution Linearity Serum	Mean: 97.7% Range: 87.1 - 113%
Dilution Linearity EDTA Plasma	Mean: 98% Range: 87.9 - 104%

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IFNγ	
Spike and Recovery Serum	Mean: 89% Range: 68.7 - 106%
Spike and Recovery EDTA Plasma	Mean: 86.4% Range: 79.7 – 91.6%
Dilution Linearity Serum	Mean: 98.6% Range: 84.6- 113%
Dilution Linearity EDTA Plasma	Mean: 103% Range: 97.6 - 116%

IL-8	
Spike and Recovery Serum	Mean: 102% Range: 87.4 - 121%
Spike and Recovery EDTA Plasma	Mean: 76.3% Range: 69.3 – 82.6%
Dilution Linearity Serum	Mean: 93.3% Range: 74.7 - 130%
Dilution Linearity EDTA Plasma	Mean: 95.3% Range: 82.2 - 107%

The Simoa Cytokine 4-Plex C (C4PC) assay kit is formulated for use on the HD-X platform. Verification and validation results for the fully automated HD-X instrument are summarized in this report.

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