# Simoa® Cytokine 4-Plex A (C4PA) Advantage PLUS Kit
## HD-X Data Sheet Item 104979

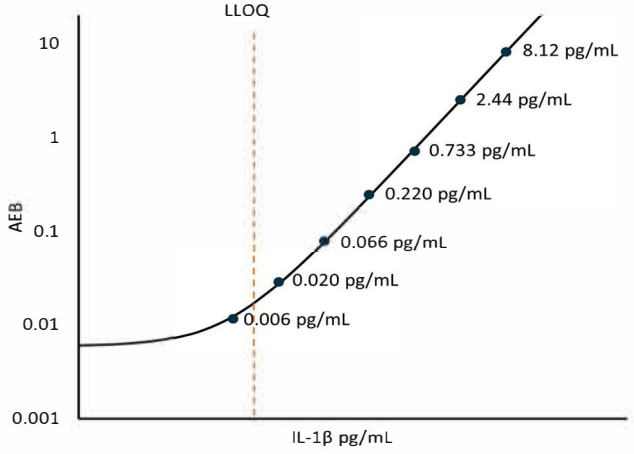
**Description**

This datasheet summarizes data from analytical validation performed at Quanterix to characterize performance of the Cytokine 4-Plex A (C4PA) 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-1β

Interleukin-1 beta (IL-1β), also known as catabolin, is a cytokine of 269 amino acids (molecular weight 31 kDa). This cytokine 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 and is involved in a variety of cellular activities, including cell proliferation, differentiation, and apoptosis. IL-1β is the most studied member of the IL-1 family of cytokines due to its role in mediating autoinflammatory diseases. Blood monocytes from patients with autoinflammatory syndromes release more processed IL-1β than cells from healthy subjects and thus likely account for the inflammation in 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.

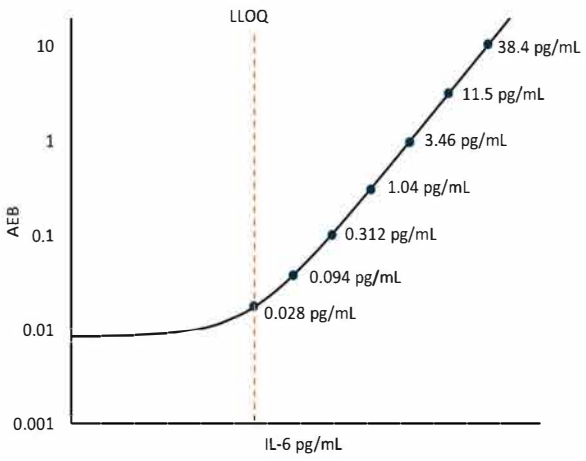
**IL-1β Curve:** Representative calibrator concentrations and Lower Limit of Quantification (LLOQ) depicted.




### 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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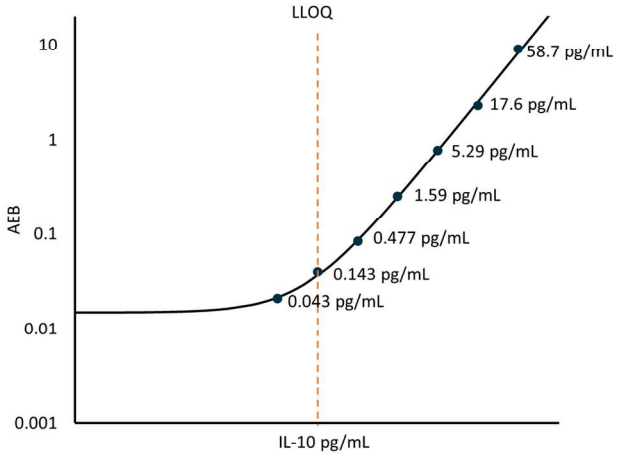
## IL-10

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-γ, IL-2, TNFα, and GM-CSF made by macrophages and regulatory T 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.

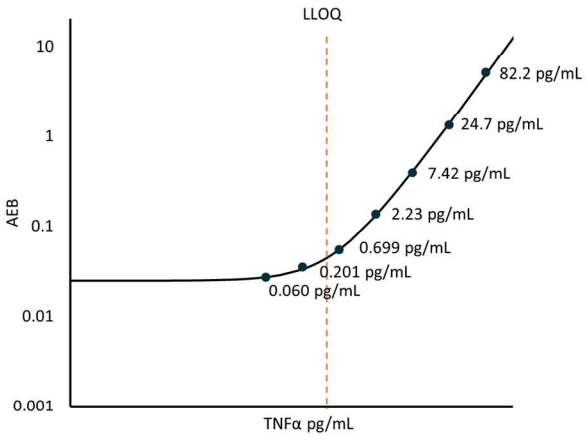
## TNFα

Human tumor necrosis factor alpha (TNFα) is a homotrimeric transmembrane protein that functions as a proinflammatory cytokine. Human TNFα is a non-glycosylated protein of 157 amino acids (17 kDa). It is produced mainly by macrophages but also by a variety of other cell types, including monocytes, neutrophils, and T cells. It is functionally known to trigger various inflammatory molecules, including other cytokines and chemokines. The TNFα soluble form facilitates various biological activities through type 1 (TNFR1) and type 2 (TNFR2) receptors. TNFR1 is expressed by all tissues and is the key signaling receptor for TNFα. 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 stems from its association with numerous disease states including rheumatoid arthritis, cancer, cachexia, and Crohn's disease.

**IL-10 Curve:** Representative calibrator concentrations and Lower Limit of Quantification (LLOQ) depicted.




**TNFα 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-1β | |
|---|---|
| Analytical LLOQ | 0.010 pg/mL<br>Pooled CV: 19%<br>Recovery: 111% |
| Functional LLOQ | Serum/EDTA Plasma (4x): 0.040 pg/mL |
| LOD | 0.002 pg/mL<br>Range: 0.0005 - 0.005 pg/mL |
| Dynamic Range | Serum/EDTA Plasma (4x): 0 - 48 pg/mL |

| IL-6 | |
|---|---|
| Analytical LLOQ | 0.028 pg/mL<br>Pooled CV: 16%<br>Recovery: 98% |
| Functional LLOQ | Serum/EDTA Plasma (4x): 0.112 pg/mL |
| LOD | 0.008 pg/mL<br>Range: 0.002 - 0.017 pg/mL |
| Dynamic Range | Serum/EDTA Plasma (4x): 0 - 160 pg/mL |

| IL-10 | |
|---|---|
| Analytical LLOQ | 0.143 pg/mL<br>Pooled CV: 13%<br>Recovery: 106% |
| Functional LLOQ | Serum/EDTA Plasma (4x): 0.572 pg/mL |
| LOD | 0.033 pg/mL<br>Range: 0.007 - 0.06 pg/mL |
| Dynamic Range | Serum/EDTA Plasma (4x): 0 - 300 pg/mL |

| TNFα | |
|---|---|
| Analytical LLOQ | 0.481 pg/mL *<br>CV at LLOQ: 20% *<br>*Pooled CV at LOQ target of 0.669 pg/mL: 9%*<br>*Recovery at LOQ target of 0.669 pg/mL: 104%* |
| Functional LLOQ | Serum/EDTA Plasma (4x): 1.92 pg/mL |
| LOD | 0.061 pg/mL<br>Range: 0.007 - 0.134pg/mL |
| Dynamic Range | Serum/EDTA Plasma (4x): 0 - 400 pg/mL |

** Using Regression analysis of %CV profiling at 2 CV.*

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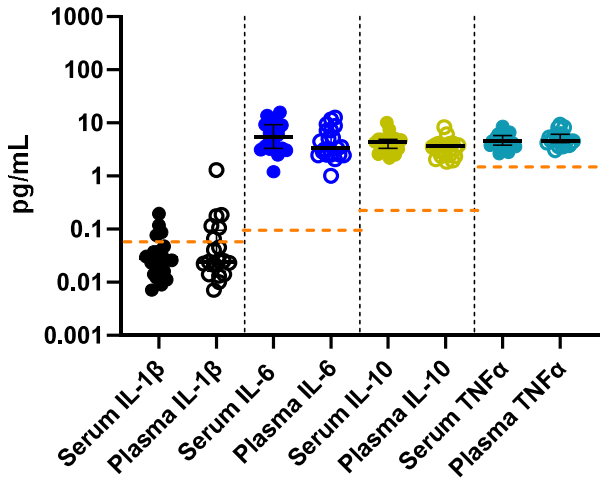
**Endogenous Sample Reading:** Concentrations (pg/mL) were determined for matched EDTA plasma (*n*=20), and serum (*n*=20) from normal human donors using the C4PA Advantage PLUS kit on HD-X. Bars depict median with interquartile range. Orange line represents functional LLOQ.




| IL-1β | | | | |
|---|---|---|---|---|
| Sample | Mean* (pg/mL) | Median* (pg/mL) | % Above LOD | % Above LLOQ |
| Serum | 0.104 | 0.087 | 95% | 25% |
| EDTA Plasma | 0.285 | 0.114 | 95% | 35% |

| IL-6 | | | | |
|---|---|---|---|---|
| Sample | Mean (pg/mL) | Median (pg/mL) | % Above LOD | % Above LLOQ |
| Serum | 6.41 | 5.20 | 100% | 100% |
| EDTA Plasma | 4.97 | 3.40 | 100% | 100% |

| IL-10 | | | | |
|---|---|---|---|---|
| Sample | Mean (pg/mL) | Median (pg/mL) | % Above LOD | % Above LLOQ |
| Serum | 4.43 | 4.40 | 100% | 100% |
| EDTA Plasma | 3.51 | 3.56 | 100% | 100% |

| TNFα | | | | |
|---|---|---|---|---|
| Sample | Mean (pg/mL) | Median (pg/mL) | % Above LOD | % Above LLOQ |
| Serum | 4.74 | 4.46 | 100% | 100% |
| EDTA Plasma | 5.18 | 4.63 | 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-1β | | | | | |
|---|---|---|---|---|---|
| Sample | Mean (pg/mL) | Within Run CV | Between Run CV | Between Lot CV | Between Instr CV |
| Control 1 | 0.315 | 5.8% | 6.8% | 0.9% | 0.7% |
| Control 2 | 18.9 | 2.9% | 4.6% | 1.2% | 1.0% |
| Panel 1 | 0.091 | 11% | 14% | 5.3% | 5.8% |
| Panel 2 | 6.30 | 4.5% | 5.5% | 2.2% | 2.6% |
| Panel 3 | 5.73 | 12% | 9.4% | 2.4% | 0.1% |
| Panel 4 | 0.097 | 7.9% | 11% | 10% | 1.6% |
| Panel 5 | 6.21 | 6.1% | 9.1% | 0.4% | 0.6% |
| Panel 6 | 7.30 | 4.8% | 14% | 0.6% | 3.6% |

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| IL-6 | | | | | |
|---|---|---|---|---|---|
| Sample | Mean (pg/mL) | Within Run CV | Between Run CV | Between Lot CV | Between Instr CV |
| Control 1 | 1.48 | 4.2% | 5.4% | 2.7% | 0.9% |
| Control 2 | 86.7 | 3.4% | 4.4% | 0.2% | 0.4% |
| Panel 1 | 2.37 | 7.6% | 14% | 2.7% | 5.4% |
| Panel 2 | 65.9 | 4.7% | 4.2% | 4.3% | 0.9% |
| Panel 3 | 57.5 | 14% | 13% | 8.3% | 1.7% |
| Panel 4 | 0.630 | 7.7% | 7.7% | 2.2% | 0.9% |
| Panel 5 | 64.5 | 5.2% | 7.9% | 8.1% | 0.9% |
| Panel 6 | 64.1 | 4.1% | 16% | 6.3% | 2.2% |

| IL-10 | | | | | |
|---|---|---|---|---|---|
| Sample | Mean (pg/mL) | Within Run CV | Between Run CV | Between Lot CV | Between Instr CV |
| Control 1 | 1.17 | 7.8% | 15% | 12% | 4.9% |
| Control 2 | 65.7 | 3.6% | 12% | 12% | 1.8% |
| Panel 1 | 1.50 | 13% | 13% | 4.0% | 1.6% |
| Panel 2 | 177 | 4.6% | 6.1% | 6.4% | 2.0% |
| Panel 3 | 149 | 14% | 12% | 0.3% | 1.5% |
| Panel 4 | 0.587 | 16% | 17% | 12% | 2.5% |
| Panel 5 | 127 | 4.3% | 6.1% | 5.4% | 1.1% |
| Panel 6 | 130 | 4.2% | 14% | 7.2% | 4.3% |

| TNFα | | | | | |
|---|---|---|---|---|---|
| Sample | Mean (pg/mL) | Within Run CV | Between Run CV | Between Lot CV | Between Instr CV |
| Control 1 | 2.55 | 9.4% | 10% | 4.5% | 4.9% |
| Control 2 | 145 | 4.1% | 4.2% | 0.5% | 1.6% |
| Panel 1 | 3.13 | 11% | 15% | 4.1% | 2.2% |
| Panel 2 | 94.4 | 6.4% | 6.0% | 2.2% | 3.9% |
| Panel 3 | 79.1 | 11% | 11% | 7.5% | 1.2% |
| Panel 4 | 1.11 | 15% | 12% | 3.8% | 7.0% |
| Panel 5 | 136 | 6.2% | 6.9% | 6.2% | 2.6% |
| Panel 6 | 137 | 5.6% | 16% | 4.4% | 5.3% |

**Spike and Recovery:** 2 serum and 2 EDTA plasma samples were spiked at high and low concentrations of IL-1β, IL-6, IL-10, and TNFα 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.

**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 32x or 64x. For valid comparison between results, it is recommended to run all samples at a consistent dilution.

| IL-1β | |
|---|---|
| Spike and Recovery Serum | Mean: 85%<br>Range: 79 – 97% |
| Spike and Recovery EDTA Plasma | Mean: 74%<br>Range: 62 – 90% |
| Dilution Linearity Serum (2X-64X) | Mean: 106%<br>Range: 96 - 130% |
| Dilution Linearity EDTA Plasma (2X-64X) | Mean: 107%<br>Range: 96 - 120% |

| IL-6 | |
|---|---|
| Spike and Recovery Serum | Mean: 92%<br>Range: 85 - 100% |
| Spike and Recovery EDTA Plasma | Mean: 79%<br>Range: 68 – 87% |
| Dilution Linearity Serum (2X-64X) | Mean: 104%<br>Range: 98 - 115% |
| Dilution Linearity EDTA Plasma (2X-64X) | Mean: 105%<br>Range: 95 - 118% |

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| IL-10 | |
|---|---|
| Spike and Recovery Serum | Mean: 93%<br>Range: 85 – 100% |
| Spike and Recovery EDTA Plasma | Mean: 79%<br>Range: 71 – 88% |
| Dilution Linearity Serum (2X-64X) | Mean: 104%<br>Range: 88 – 141% |
| Dilution Linearity EDTA Plasma (2X-64X) | Mean: 96%<br>Range: 88 – 107% |

| TNFα | |
|---|---|
| Spike and Recovery Serum | Mean:79%<br>Range: 71 – 88% |
| Spike and Recovery EDTA Plasma | Mean: 73%<br>Range: 62 – 84% |
| Dilution Linearity Serum (2X-32X) | Mean: 120%<br>Range: 107 - 133% |
| Dilution Linearity EDTA Plasma (2X-32) | Mean: 115%<br>Range: 110 - 121% |

The Simoa Cytokine 4-Plex A (C4PA) 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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