Quanterix™
The Science of Precision Health

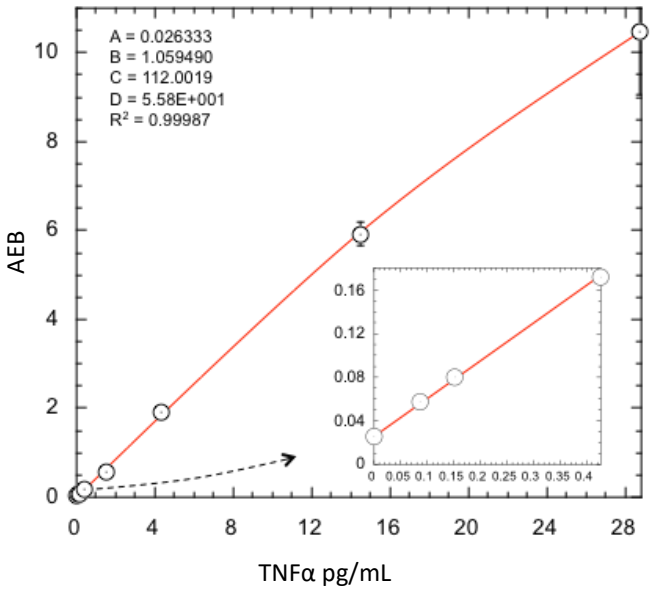
Simoa® Cytokine 3-Plex B (TNFα, IL-6, IL-17A) HD-1/HD-X Data Sheet

Item 101319

Description

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.

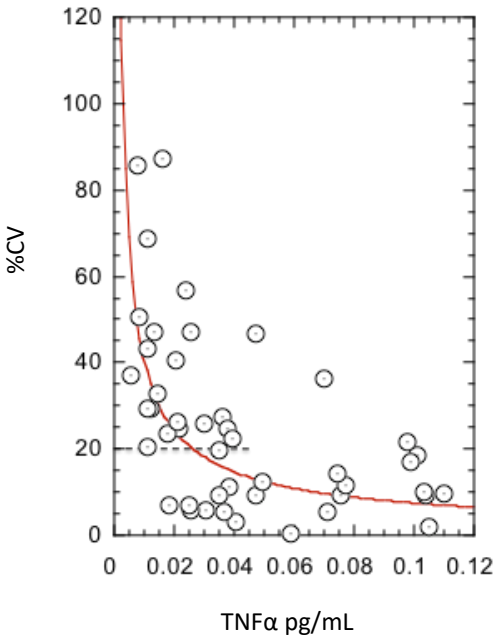
Calibration Curve: Four-parameter curve fit parameters are depicted.



Limit of Detection (LOD), Lot A: Calculated as 2.5 standard deviations from the mean of background signal read back on each calibration curve over 15 runs across 2 instruments.

Limit of Detection (LOD), Lot B: Calculated as 2.5 standard deviations from the mean of background signal read back on each calibration curve over 6 runs across 3 instruments.

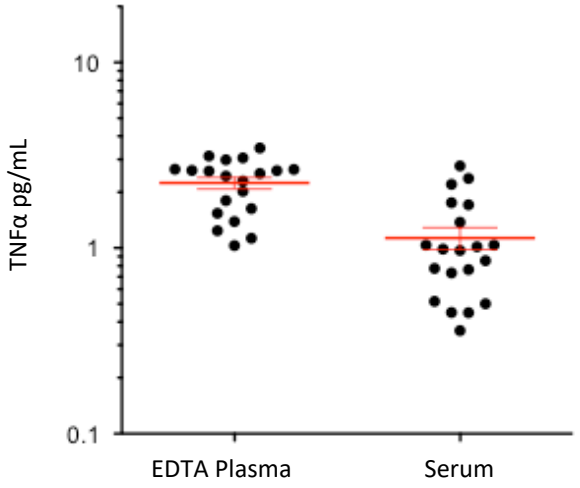
Sample Dose CV Profile: Triplicate measurements of diluted serum samples assayed over multiple runs (47 measurements). LLOQ determined as the concentration at which %CV exceeds 20% according to the power equation fit of the data.



LLOQ (Lot A)	**0.026 pg/mL**
LOD (Lot A)	**0.021 pg/mL** SD 0.0172 pg/mL
LOD (Lot B)	**0.030 pg/mL** SD 0.0305 pg/mL
Dynamic range (serum and plasma)	0–112 pg/mL
Diluted Sample volume*	100 µL per measurement
Tests per kit	96

***See Kit Instruction for details**

Endogenous Sample Reading: Healthy donor matched EDTA plasma (n=20) and serum (n=20) were measured. Error bars depict mean and SEM.



Sample Type	Median TNFα pg/mL	% Above LOD
Serum	0.98	100%
Plasma	2.48	100%

Precision: Six samples consisting of three serum-based panels, one plasma-based panel, and two TNFα controls were assayed in replicates of three at two separate times per day for seven days using a single lot of reagents. Analysis of variance (fully nested ANOVA) results are summarized in the following table.

Sample	Mean (pg/mL)	Within run CV	Between run CV	Between day CV
Control 1	1.98	6.2%	3.1%	2.0%
Control 2	47.9	6.3%	5.1%	0.0%
Panel 1	1.36	10.3%	5.4%	3.5%
Panel 2	3.70	6.7%	0.0%	8.8%
Panel 3*	4.21	7.0%	0.0%	6.6%
Panel 4	23.9	7.9%	5.6%	9.8%

*Plasma

Spike and Recovery: TNFα spiked into 3 serum samples at 3 and 60 pg/mL.

Admixture Linearity: High TNFα sample admixed with low TNFα sample, mean of 12 levels.

Dilution Linearity: Serum sample diluted 2x serially from MRD (4x) to 256x with sample diluent.

Spike and Recovery (Serum)	**Mean = 63.5%** Range: 43.7–84.9%
Admixture Linearity	**Mean = 93.0%** Range: 79.0–101.4%
Dilution Linearity (256x)	**Mean = 124.5%** Range: 115.7–134.9%

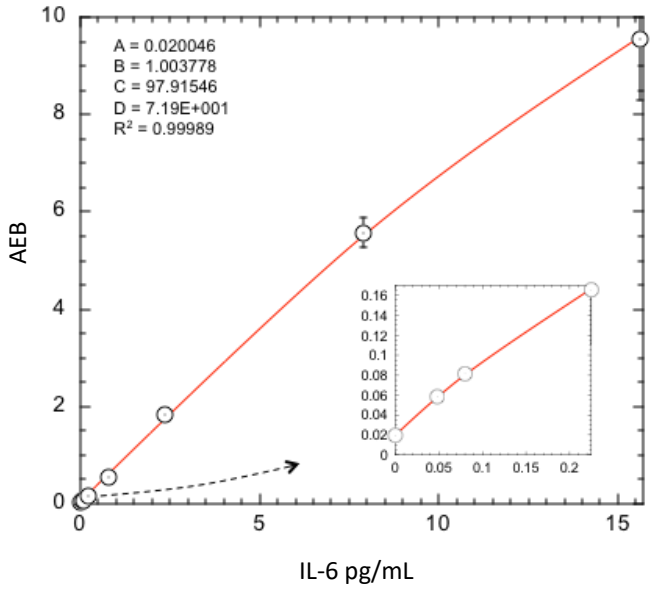
Simoa® Cytokine 3-Plex B (TNFα, IL-6, IL-17A) HD-1/HD-X Data Sheet

Item 101319

Description

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 22k-28k dalton 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.

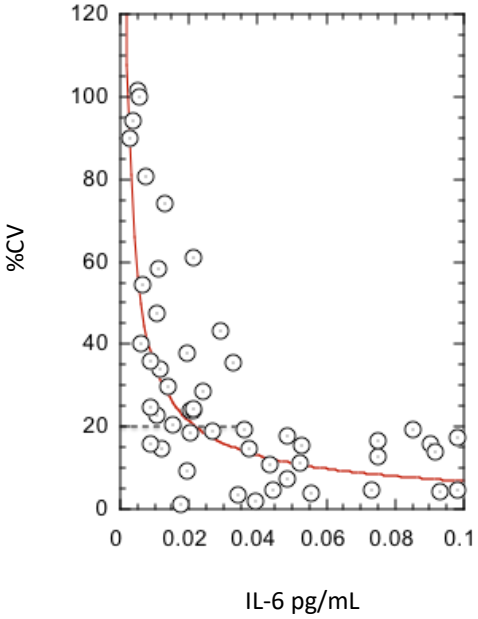
Calibration Curve: Four-parameter curve fit parameters are depicted.



Limit of Detection (LOD), Lot A: Calculated as 2.5 standard deviations from the mean of background signal read back on each calibration curve over 15 runs across 2 instruments.

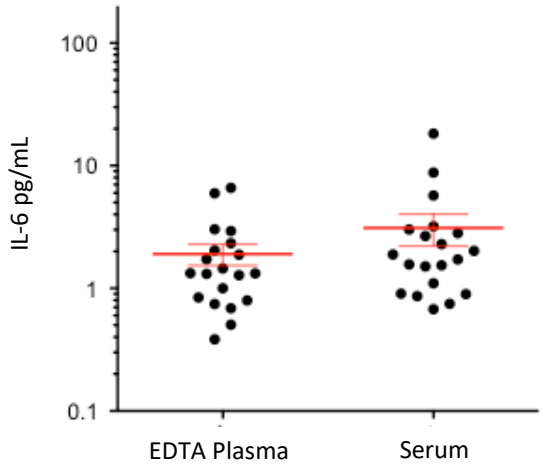
Limit of Detection (LOD), Lot B: Calculated as 2.5 standard deviations from the mean of background signal read back on each calibration curve over 6 runs across 3 instruments.

Sample Dose CV Profile: Triplicate measurements of diluted serum samples assayed over multiple runs (50 measurements). LLOQ determined as the concentration at which %CV exceeds 20% according to the power equation fit of the data.



LLOQ (Lot A)	**0.023 pg/mL**
LOD (Lot A)	**0.011 pg/mL** SD 0.0084 pg/mL
LOD (Lot B)	**0.013 pg/mL** SD 0.0093 pg/mL
Dynamic range (serum and plasma)	0–60 pg/mL
Diluted Sample volume*	100 µL per measurement
Tests per kit	96

Endogenous Sample Reading: Healthy donor matched EDTA plasma (n=20) and serum (n=20) were measured. Error bars depict mean and SEM.



Sample Type	Median IL-6 pg/mL	% Above LOD
Serum	1.80	100%
Plasma	1.33	100%

Precision: Six samples consisting of three serum-based panels, one plasma-based panel, and two IL-6 controls were assayed in replicates of three at two separate times per day for seven days using a single lot of reagents. Analysis of variance (fully nested ANOVA) results are summarized in the following table.

Sample	Mean (pg/mL)	Within run CV	Between run CV	Between day CV
Control 1	0.974	5.5%	3.0%	2.5%
Control 2	23.8	7.1%	4.8%	0.0%
Panel 1	16.8	7.6%	7.2%	0.0%
Panel 2	1.10	10.4%	0.0%	7.1%
Panel 3*	2.14	7.2%	0.0%	2.5%
Panel 4	1.18	6.9%	7.4%	0.0%

*Plasma

Spike and Recovery: IL-6 spiked into 3 serum samples at 1.5 and 30 pg/mL.

Admixture Linearity: High IL-6 sample admixed with low IL-6 sample, mean of 12 levels.

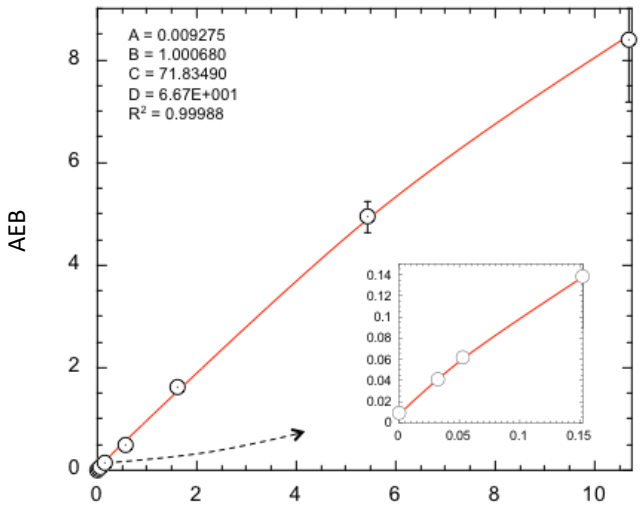
Dilution Linearity: Serum sample diluted 2x serially from MRD (4x) to 256x with sample diluent.

Spike and Recovery (Serum)	**Mean = 87.2%** Range: 76.9–96.0%
Admixture Linearity	**Mean = 93.0%** Range: 85.1–101.1%
Dilution Linearity (256x)	**Mean = 102.8%** Range: 92.6–108.2%

Description

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). 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. 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.

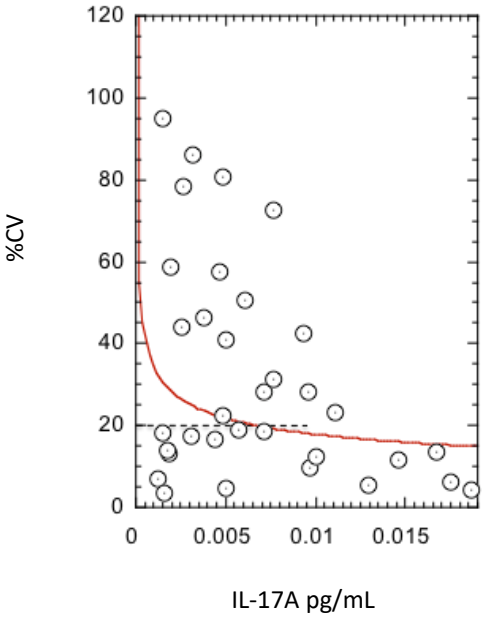
Calibration Curve: Four-parameter curve fit parameters are depicted.



Limit of Detection (LOD), Lot A: Calculated as 2.5 standard deviations from the mean of background signal read back on each calibration curve over 15 runs across 2 instruments.

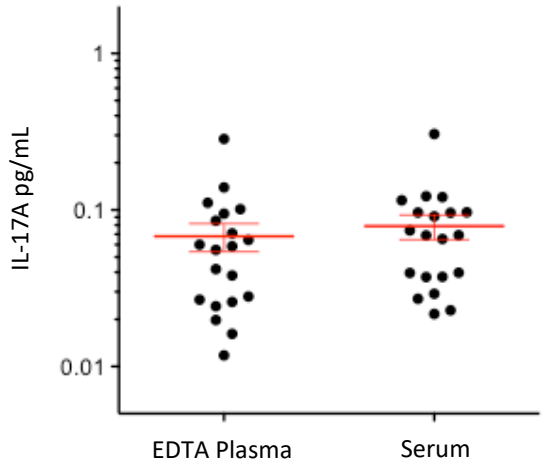
Limit of Detection (LOD), Lot B: Calculated as 2.5 standard deviations from the mean of background signal read back on each calibration curve over 6 runs across 3 instruments.

Sample Dose CV Profile: Triplicate measurements of diluted serum samples assayed over multiple runs (37 measurements). LLOQ determined as the concentration at which %CV exceeds 20% according to the power equation fit of the data.



LLOQ (Lot A)	**0.0068 pg/mL**
LOD (Lot A)	**0.0047 pg/mL** SD 0.0033 pg/mL
LOD (Lot B)	**0.0059 pg/mL** SD 0.0042 pg/mL
Dynamic range (serum and plasma)	0–40 pg/mL
Diluted Sample volume*	100 µL per measurement
Tests per kit	96

Endogenous Sample Reading: Healthy donor matched EDTA plasma (n=20) and serum (n=20) were measured. Error bars depict mean and SEM.



Sample Type	Median IL-17A pg/mL	% Above LOD
Serum	0.069	100%
Plasma	0.057	100%

Precision: Six samples consisting of three serum-based panels, one plasma-based panel, and two IL-17A controls were assayed in replicates of three at two separate times per day for seven days using a single lot of reagents. Analysis of variance (fully nested ANOVA) results are summarized in the following table.

Sample	Mean (pg/mL)	Within run CV	Between run CV	Between day CV
Control 1	0.556	5.2%	5.0%	0.0%
Control 2	13.7	7.1%	4.3%	0.0%
Panel 1	0.497	9.5%	4.6%	3.4%
Panel 2	0.388	6.7%	2.8%	3.9%
Panel 3*	1.11	6.8%	4.6%	0.0%
Panel 4	6.45	8.3%	4.4%	5.5%

*Plasma

Spike and Recovery: IL-17A spiked into 3 serum samples at 1.2 and 20 pg/mL.

Admixture Linearity: High IL-17A sample admixed with low IL-17A sample, mean of 12 levels.

Dilution Linearity: Serum sample diluted 2x serially from MRD (4x) to 256x with sample diluent.

Spike and Recovery (Serum)	**Mean = 59.8%** Range: 38.8–75.0%
Admixture Linearity	**Mean = 89.6%** Range: 73.1–102.5%
Dilution Linearity (256x)	**Mean = 108.6%** Range: 99.7–117.3%