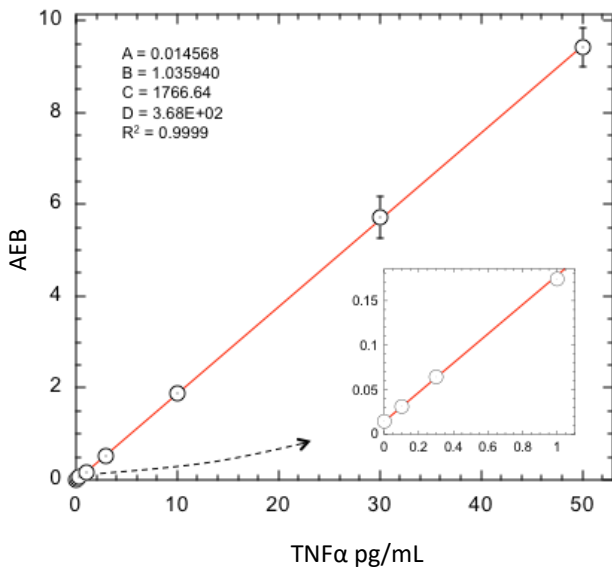


Description

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.



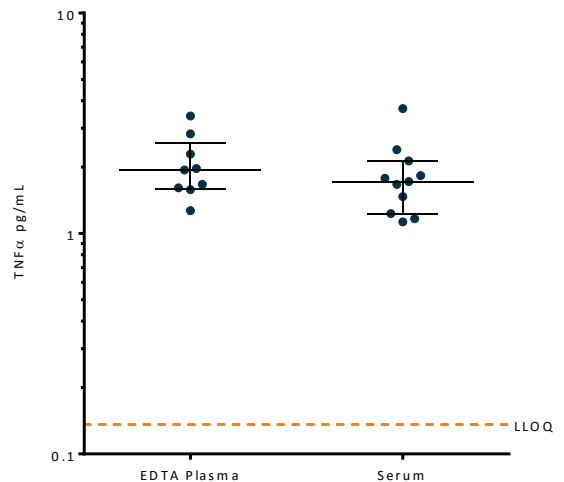
Lower Limit of Quantification (LLOQ): Triplicate measurements of serially diluted calibrator were read back on the calibration curve over 2 reagent lots across 3 instruments (12 runs total).

Limit of Detection (LOD): Calculated as 2.5 standard deviations from the mean of background signal read back on each calibration curve over 2 reagent lots across 3 instruments (12 runs total).

LLOQ	0.034 pg/mL
LOD	0.016 pg/mL SD 0.0186 pg/mL
Dynamic range (serum and plasma)	0–200 pg/mL
Diluted Sample volume*	100 µL per measurement
Tests per kit	192

*See Kit Instruction for details

Endogenous Sample Reading: Healthy donor matched EDTA plasma (n=9) and serum (n=11) were measured. Error bars depict mean and SEM.



Sample Type	Median TNFα pg/mL	% Above LOD
Serum	1.78	100%
EDTA Plasma	1.94	100%

Precision: Five samples consisting of three serum-based panels and two TNFα controls were assayed in replicates of three at two separate times per day for five days using a single lot of reagents and calibrators. 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
Control 1	2.77	3.4%	4.1%	0.0%
Control 2	29.9	2.8%	4.8%	0.0%
Panel 1	1.71	4.9%	9.1%	0.0%
Panel 2	2.15	5.5%	9.2%	0.0%
Panel 3	43.4	3.5%	0.4%	1.0%

Spike and Recovery: TNFα spiked into 4 serum samples at 2 levels.

Admixture Linearity: High TNFα serum sample admixed with low TNFα sample, mean of 10 levels.

Dilution Linearity: 1 spiked serum sample was diluted 2x serially from MRD (4x) to 64x with Sample Diluent.

Lot Consistency: 5 samples tested with 2 reagent lots across 2 runs x 3 instruments.

Spike and Recovery (Serum)	Mean = 77.9% Range: 68.2–87.1%
Admixture Linearity	Mean = 108%
Dilution Linearity (64x)	Mean = 121% Range: 101–139%
Lot Consistency	Mean Difference = -2.7% Range: 1.34–41.1 pg/mL

The Simoa TNFα Discovery assay kit is formulated for use on the SR-X®, HD-1, or HD-X® platform. Data in this document was obtained from runs on the HD-1 platform unless otherwise noted. Some differences in performance claims between SR-X and HD-1/HD-X may be observed when comparing datasheets for these platforms. This may be due to experiments run at different time-points with different reagent lots and different samples, or it may be due to minor differences in antibody and analyte behavior in the different assay formats.