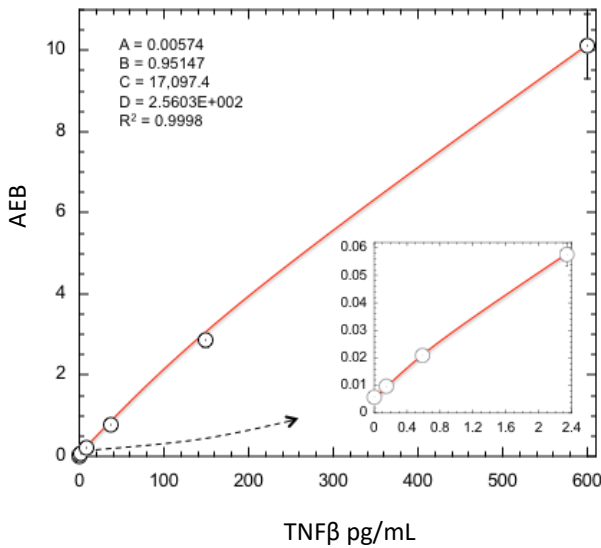


Description

Tumor Necrosis Factor-beta (TNFβ), also known as Lymphotoxin-alpha (LT-α), is a member of the TNF superfamily. Human TNFβ is a 22 kDa protein that shares 73% amino acid sequence identity with mouse and rat TNFβ. Secreted TNFβ can form homotrimers that bind and activate multiple receptors. With membrane-localized LT-β, LT-α /TNFβ forms heterotrimers to bind and activate the LT-β R/TNFRSF3. TNF was initially identified for inducing necrosis in experimental cancers, but then a tumour promoting role was identified. TNFβ is expressed in activated T- and B- lymphocytes and contributes to autoimmune disease. TNFβ is found to mediate lymph node development, inflammation, and immune function.

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



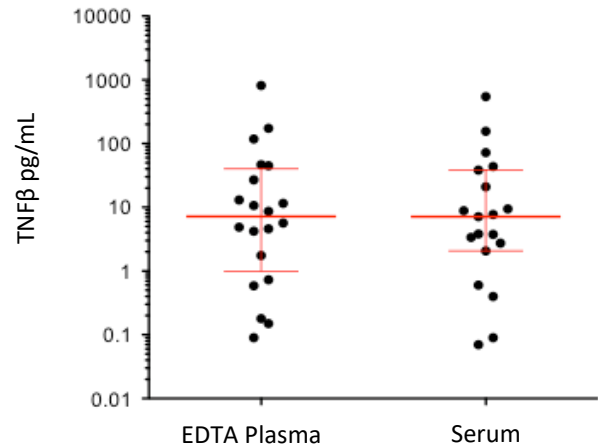
Lower Limit of Quantification (LLOQ): Triplicate measurements of serially diluted calibrator were read back on the calibration curve over 1 reagent lot across 3 instruments (5 runs total).

Limit of Detection (LOD): Calculated as 2.5 standard deviations from the mean of background signal read back on each calibration curve over 1 reagent lot across 3 instruments (5 runs total).

LLOQ	0.150 pg/mL pooled CV 11% mean recovery 107%
LOD	0.052 pg/mL range 0.0174–0.0833 pg/mL
Dynamic range (serum and plasma)	0–2400 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=20) and serum (n=19) were measured. Error bars depict median with interquartile range.



Sample Type	Median TNFβ pg/mL	% Above LOD
Serum	7.14	80%
EDTA Plasma	7.17	85%

Precision: Representative precision was estimated with repeated assay of serum and plasma panels using 3 instruments and one reagent lot. Within-run and between-run CVs are depicted in the following table. Within-run CVs reflect average CVs across 5 experiments of 3 replicates each.

Sample	Mean (pg/mL)	Within run CV	Between run CV
Serum Panel 1	2.03	9.3%	18.1%
Serum Panel 2	18.9	4.2%	7.7%
Plasma Panel 1	47.5	4.3%	11.2%

Spike and Recovery: TNFβ spiked into 2 serum samples and 2 plasma samples at 2 levels.

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

Dilution Linearity: Endogenous serum sample diluted 2x serially from MRD (4x) to 256x with Sample Diluent.

Spike and Recovery (Serum/Plasma)	Mean = 81% Range: 68.3–95.1%
Admixture Linearity	Mean = 94% Range: 87–102%
Dilution Linearity (256x)	Mean = 74% Range: 66–84%