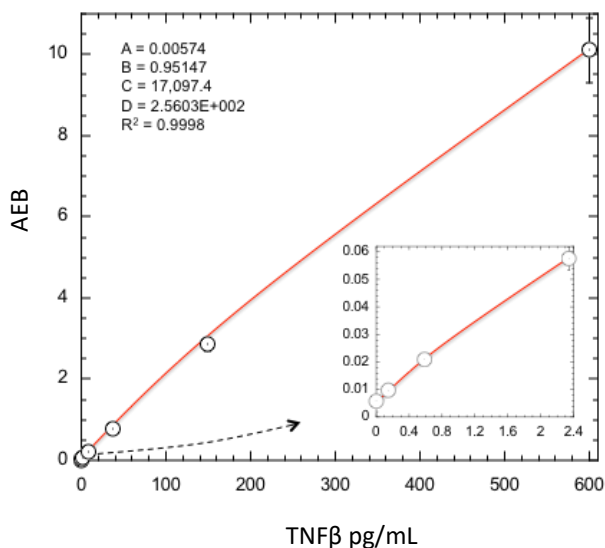


**Description**

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.



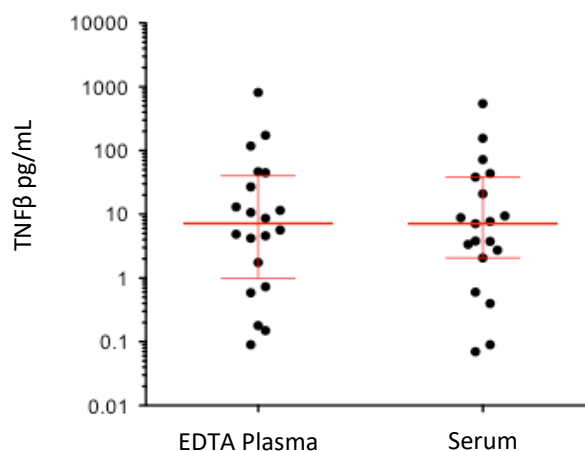
**Lower Limit of Quantification (LLOQ):** Triplicate measurements of serially diluted calibrator were read back on the calibration curve over 1 reagent lot across 3 instruments (5 runs total).

**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).

|   |   |
|---|---|
| <b>LLOQ</b>                             | <b>0.150 pg/mL</b><br>pooled CV 11%<br>mean recovery 107% |
| <b>LOD</b>                              | <b>0.052 pg/mL</b><br>range 0.0174–0.0833 pg/mL           |
| <b>Dynamic range (serum and plasma)</b> | 0–2400 pg/mL  |
| <b>Diluted Sample volume*</b>           | 100 μL<br>per measurement                                 |
| <b>Tests per kit</b>                    | 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.

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