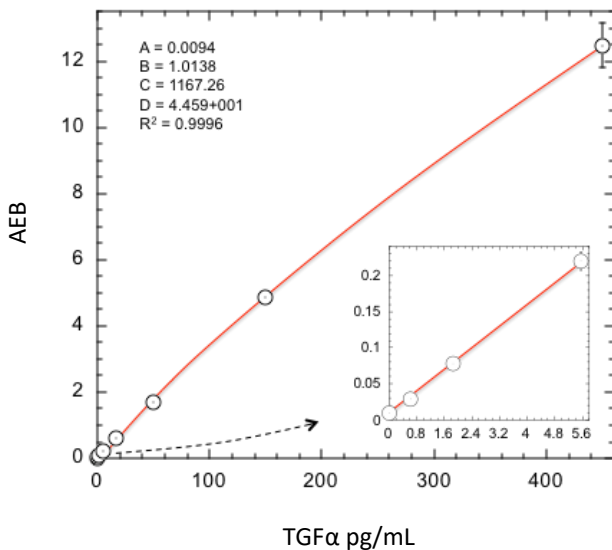


Description

Transforming Growth Factor alpha (TGFα) is a 6 kDa polypeptide mitogen. Its precursor protein is a 160 amino acid integral membrane glycoprotein that is cleaved by a protease to produce the 50 amino acid mature TGFα. TGFα is produced by keratinocytes, macrophages, hepatocytes, and platelets; its synthesis is stimulated by viral infection. In mammary tissue, synthesis is stimulated by estrogen. Abundant TGFα expression is seen in transformed cells and tumors. It is expressed in modest amounts in non-transformed cells during development of mammalian embryos. TGFα is a ligand for EGFR and activates the EGFR signaling pathway promoting tumor progression by triggering downstream signaling molecules like AKT and MAPK. Studies have shown that activation of TGFα-EGFR signaling in primary colon tumors contributes to the spread of tumor cells to the lymph nodes and liver. It has also been shown that secretion of TGFα by NSCLC cells activates EGFR in tumor and tumor associated endothelial cells and EGFR signaling pathways in both tumor cells and tumor associated endothelial cells.

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



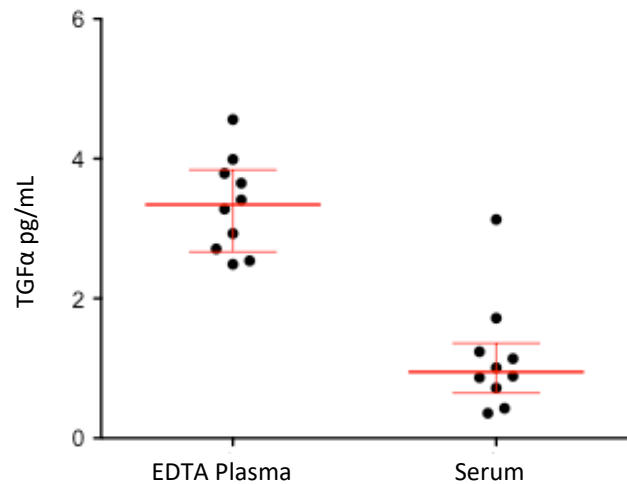
Lower Limit of Quantification (LLOQ): Triplicate measurements of serially diluted calibrator were read back on the calibration curve over 1 reagent lot across 3 instruments (5 runs total).

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 | 0.207 pg/mL pooled CV 17.6% mean recovery 111% |
| LOD | 0.031 pg/mL range 0.016–0.048 pg/mL |
| Dynamic range (serum and plasma) | 0–900 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=10) and serum (n=10) were measured. Error bars depict median with interquartile range.



| Sample Type | Median TGFα pg/mL | % Above LOD |
|-------------|-------------------|-------------|
| Serum | 0.95 | 100% |
| EDTA Plasma | 3.34 | 100% |

Precision: Representative precision was estimated with repeated assay of serum and plasma panels using three instruments and one reagent lot. Within-run and between-run CVs across the three instruments 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 | 1.12 | 8.5% | 11.2% |
| Plasma Panel 2 | 1.86 | 3.4% | 12.1% |
| Plasma Panel 3 | 1.57 | 5.0% | 12.8% |

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

Dilution Linearity: Spiked serum and plasma samples were diluted 2x serially from MRD (2x) to 128x with Sample Diluent.

| | |
|-----------------------------------|---|
| Spike and Recovery (Serum) | Mean = 88.9% Range: 67.2–113% |
| Dilution Linearity (128x) | Mean = 112.5% Range: 100.4–126.9% |