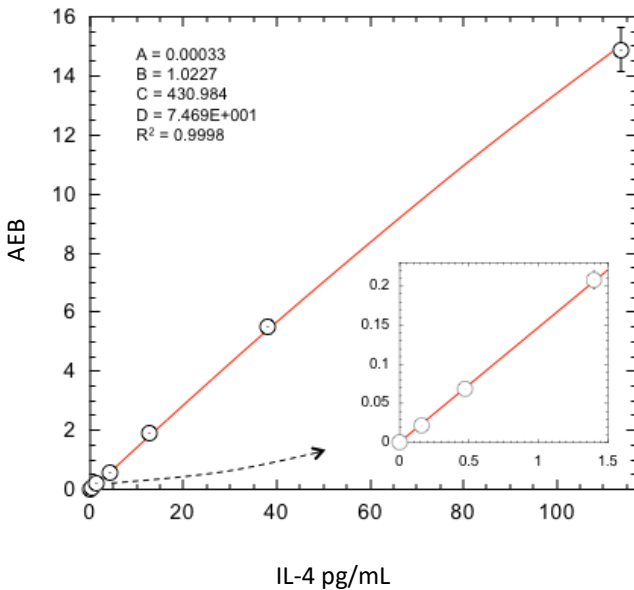## Description

Human Interleukin-4 (IL-4) is a monomeric cytokine, approximately 13-18 kDa, expressed by Th2-biased CD4+ T cells, mast cells, basophils, and eosinophils. IL-4 has a compact, globular fold (similar to other cytokines) stabilized by 3 disulphide bonds. Mature human IL-4 shares 55%, 39%, and 43% amino acid sequence identity with bovine, mouse, and rat IL-4, respectively. By binding to IL-4 receptor or receptor complex, IL-4 has many biological functions. It promotes cell proliferation, survival, and immunoglobulin class switch to IgG4 and IgE in human B cells, acquisition of the Th2 phenotype by naïve CD4+ T cells, priming and chemotaxis of mast cells, eosinophils, and basophils, and the proliferation and activation of epithelial cells. IL-4 plays an important role in the development of allergic inflammation and asthma.

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

A = 0.00033
B = 1.0227
C = 430.984
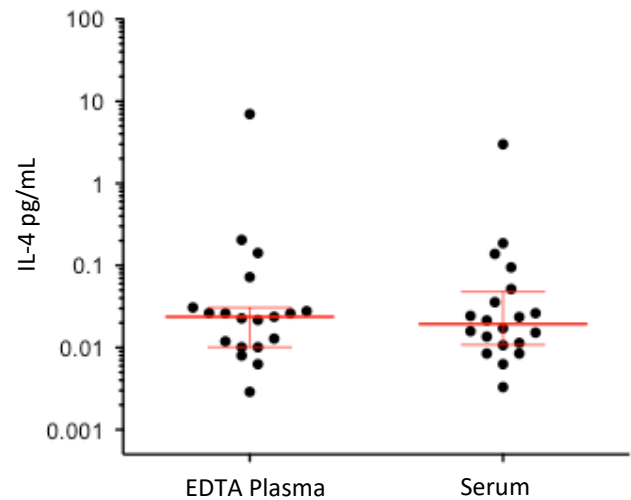D = 7.469E+001
R^2 = 0.9998

IL-4 pg/mL

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).

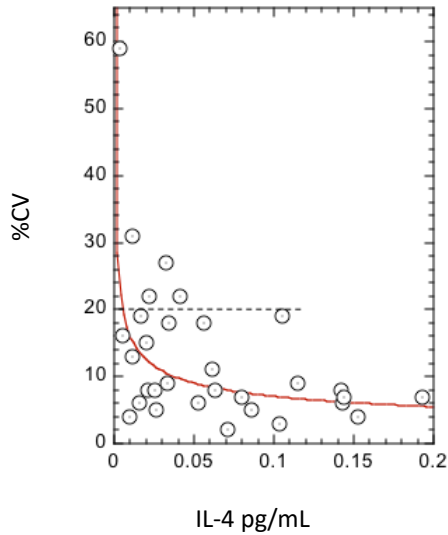|	
LLOQ	**0.039 pg/mL** pooled CV 15.4% mean recovery 110%
LOD	**0.005 pg/mL** range 0.0004–0.017 pg/mL
Dynamic range (serum and plasma)	0–200 pg/mL
Diluted Sample volume*	100 µL per measurement
Tests per kit	96

*See Kit Instruction for details

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

Sample Type	Median IL-4 pg/mL	% Above LOD
EDTA Plasma	0.024	95%
Serum	0.019	95%

Sample Dose CV Profile: Triplicate measurements of diluted serum samples assayed over multiple runs (30 measurements).

IL-4 pg/mL

Precision: Four samples consisting of two serum-based panels and two IL-4 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 CV
Control 1	8.98	3.5%	3.5%	0.0%
Control 2	92.7	5.2%	4.5%	4.1%
Panel 1	6.26	3.9%	4.8%	0.0%
Panel 2	67.7	5.5%	10.9%	0.0%

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

Admixture Linearity: High IL-4 serum sample admixed with low IL-4 sample, mean of 10 levels.

Dilution Linearity: Spiked plasma sample diluted 2x serially from MRD (2x) to 512x with Sample Diluent.

Spike and Recovery (Serum)	Mean = 83.1% Range: 64.7- 93.4%
Admixture Linearity	Mean = 93.0% Range: 77.8 - 102%
Dilution Linearity (512x)	Mean = 110% Range: 102 - 118%