Performance Characterization of a High Sensitivity Human Interleukin-22 ELISA Kit in Healthy Serum, Patient Serum and Plasma Samples

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A Sub-pg/ml IL-22 ELISA Assay for Endogenous IL-22 Quantification in Healthy & Disease Sera

- Measures Free Endogenous IL-22 as low as 0.78 pg/ml
- Accurate Quantification of Human IL-22 in Disease and Healthy Donor Sera

Sample Type	n	Mean IL-22 (pg/ml)	IL-22 Range (pg/ml)	%CV
NHS	24	1.84	0.80-5.43	5.6
Sodium Citrate Plasma	7	2.54	1.06-5.92	4.5
Atopic Dematitis	10	14.77	2.3-32.16	2.1
Psoriasis	10	5.13	1.25-25.99	3.8
Rheumatoid Arthritis	10	5.58	1.98-9.47	3.7
Systemic Lupus Erythematosus	10	3.13	1.55-9.22	4.7

Levels of Human IL-22 Quantified in Normal Human Sera (NHS), Sodium Citrate Plasma from Healthy Donors, Disease State Sera from Atopic Dermatitis (AD), Psoriasis, Rheumatoid Arthritis (RA), and Systemic Lupus Erythematosus (SLE)

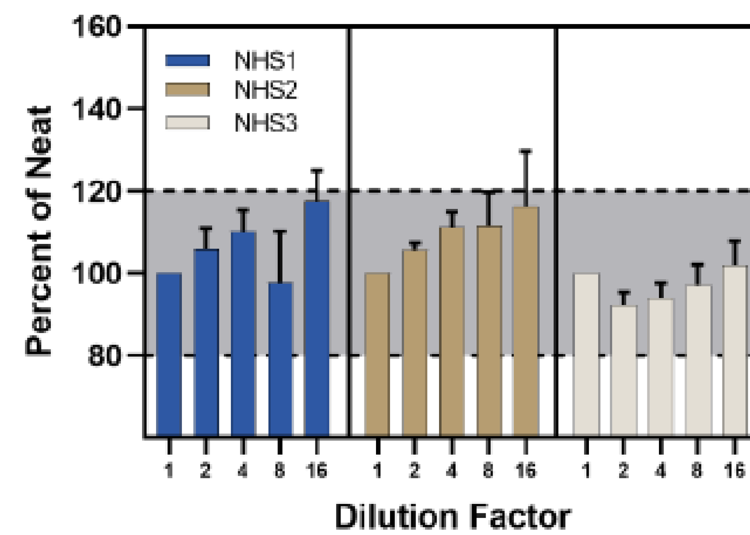

Figure 1. Parallelism of Normal Human Serum (NHS). Three NHS samples with high levels of endogenous IL-22 were assayed in duplicate to assess reliable quantification after dilution within the standard curve range. Endogenous samples were diluted in Standard Diluent of the kit to a 16-fold dilution. All samples fell within 100 ± 20% of the neat value. Error bars indicate the standard deviation.

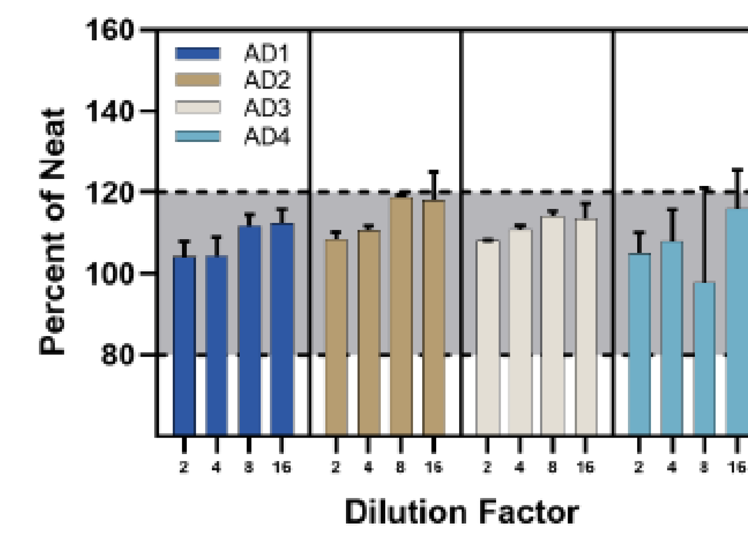

Figure 2. Parallelism of Disease State Serum. Four *Atopic Dermatitis* (AD) serum samples with high levels of endogenous IL-22 were assayed in duplicate to assess reliable quantification after dilution within the standard curve range. Samples were diluted in Standard Diluent of the kit down to a 16-fold dilution. All samples fell within 100 ± 20% of the neat value. Error bars indicate the standard deviation.

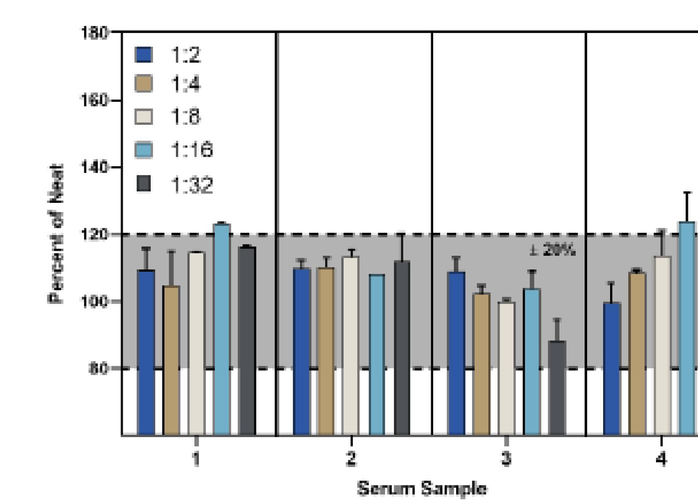

Figure 3. Linearity of Normal Human Serum (NHS). Four NHS with low levels of endogenous IL-22 were spiked with a known concentration of recombinant IL-22. Samples were diluted 2-fold in Standard Diluent of the kit to assess reliable quantification after dilution within the standard curve. Samples were diluted down to a 32-fold dilution, and endogenous levels were subtracted from dilution-corrected backfit concentrations. Error bars indicate the standard deviation.

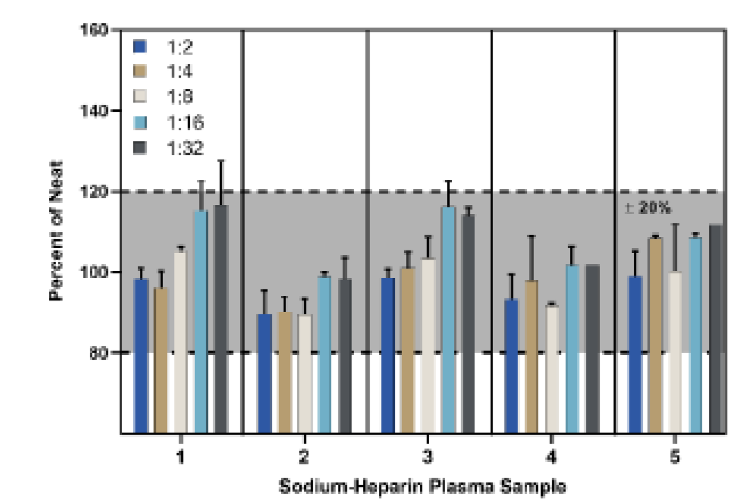

Figure 4. Linearity of Normal Sodium-Heparin Plasma. Five Sodium-Heparin Plasma samples from Healthy Donors with low endogenous levels of IL-22 were spiked with low with a known concentration of IL-22. Samples were diluted 2-fold in Standard Diluent of the kit to assess reliable quantification after dilution within the standard curve. Samples were diluted down to a 32-fold dilution, and endogenous levels were subtracted from dilution-corrected backfit concentrations. Error bars indicate the standard deviation.

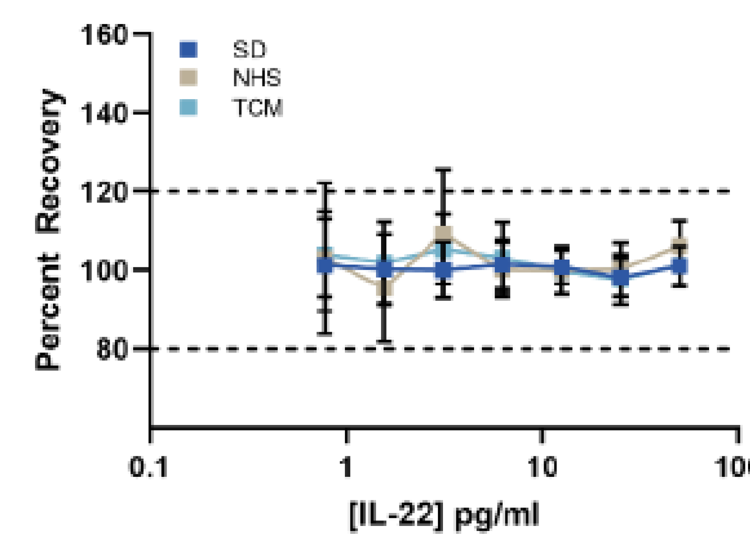

Figure 5. Percent Recovery of IL-22 in Various Matrices. Percent recoveries of Human IL-22 standard from the kit at different concentrations for various matrices. Standard Diluent curve was used a reference for calculating the percent recovery of both the Normal Human Serum (NHS) and Tissue Culture Media (TCM) curves. Endogenous levels of IL-22 were subtracted to calculate the corrected backfit concentrations of the standard. Standard curves were assayed in triplicate. Figure shows the mean of 9 runs.

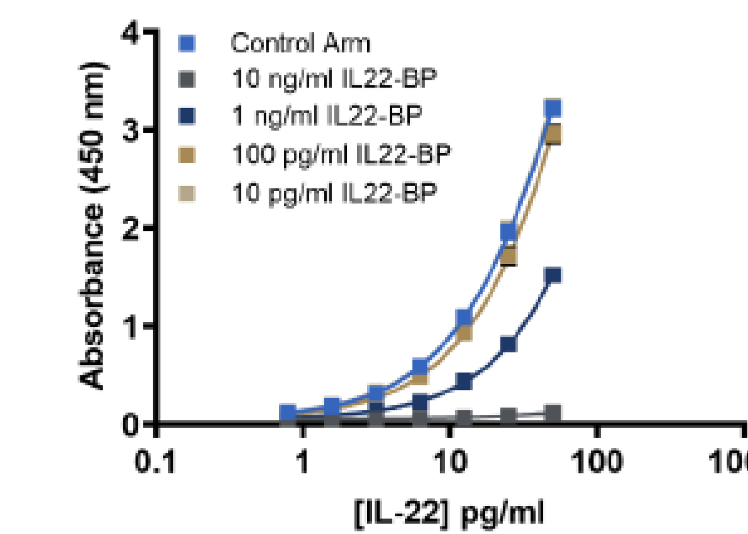

Figure 6. Representative Standard Curve in the presence of IL-22 Binding Protein (IL22-BP). Various concentrations of IL-22 standard were pre-incubated in both the absence and presence of IL22-BP. Pre-incubations were made in either Standard Diluent of the kit or Calibrator Diluent. Incubation with high IL22-BP concentrations of 10 ng/ml and 1 ng/ml of active IL22-BP interfered with detection of IL-22.

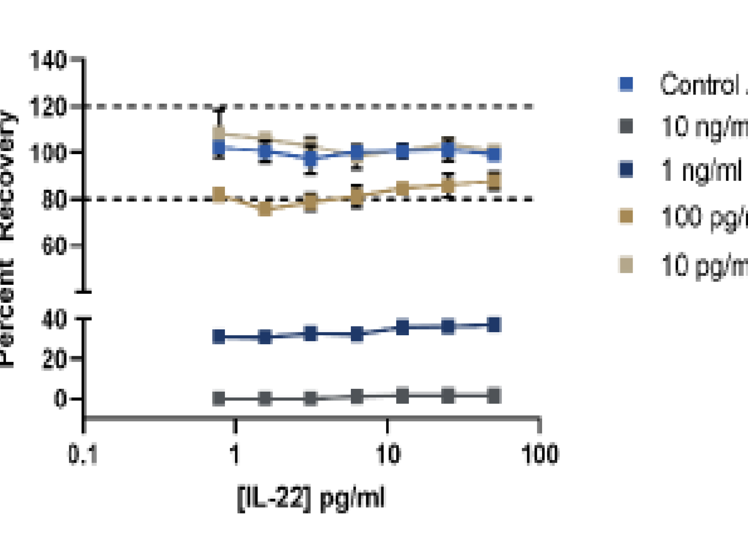

Figure 7. IL-22 Recovery in the presence of IL-22 Binding Protein (IL22-BP). Pre-incubations were made in either Standard Diluent or Calibrator Diluent. All curves of the various concentrations of binding protein were backfitted against the standard without any IL22-BP, which was used to interpolate the concentrations of IL-22 and calculate the percent recovery of the curves from the expected value.

	Intermediate Precision				
Sample	1	2	3	4	5
n	27	27	27	27	27
Mean (pg/ml)	31.93	7.76	3.43	5.30	6.45
Standard Deviation	4.34	1.24	0.42	0.62	0.94
%CV	13.60	16.00	12.30	11.80	14.60

Figure 8. Assay Intermediate Precision. Five Normal Human Serum samples with different endogenous levels were tested over 27 assays by 7 operators using 3 different lots of components.

INTRODUCTION

Interleukin-22 (IL-22) is a member of the IL-10 family of cytokines.[1] Other names include IL-TIF and Zcyto 18. IL-22 is produced by a variety of cells including TH1, TH17, TH22 T-cells, NKT cells, ILC3, neutrophils, and macrophages.[2] IL-22 targets primarily non-hemopoietic cells such as hepatocyte and epithelial cells. IL-22 has both protective effects, such as hepatocyte and epithelial cell survival, and pro-inflammatory effects. It is also implicated in autoimmune diseases such as Systemic Lupus Erythematosus (SLE), Rheumatoid Arthritis (RA), and Psoriasis.[3] IL-22 is often considered a hallmark of IL-17 driven immune responses.[4] Accurate and precise quantitation of IL-22 should be important to gain further understanding of its immunological role and to develop additional treatments for autoimmune and other illnesses.

A high sensitivity Human IL-22 was characterized for precision, specificity in the presence of IL-22 Binding Protein (IL22-BP), and limits of detection of endogenous IL-22 in healthy and disease state sera. Matrix effects were evaluated using spike recovery, Parallelism, and Linearity of dilution studies.

SUMMARY

VeriKine High Sensitivity (VeriKine-HS) Human Interleukin-22 (IL-22) ELISA kit (Cat. No. 41701-1) accurately quantifies to low pg/ml levels of IL-22 in Disease Sera, Healthy Donor Serum and Plasma, and Tissue Culture Media. Samples of each of these matrices may be evaluated for unknown concentrations of IL-22 within the assay range in bioanalysis studies.

Pre-Incubation in the presence of IL-22 Binding Protein (IL22-BP) at concentration ≥ 1 ng/ml interferes with the assay, suggesting that this ELISA assay detects and measures free/unbound Human IL-22.

The VeriKine-HS Human IL-22 ELISA exhibits good parallelism and linearity-of-dilution performance both in normal human serum (NHS) and in disease sera, such as that from *Atopic Dermatitis* (AD) patients. The assay offers precise results over multiple levels of dilutions in complex matrices.

MATERIALS AND METHODS

VeriKine High Sensitivity Human IL-22 ELISA (Catalog No. 41701, PBL Assay Science, Piscataway, NJ). This ELISA has an assay range of 0.78 - 50 pg/ml. Human IL-22 binds to plates coated with capture antibody and is detected by a biotinylated secondary antibody followed by streptavidin conjugated to horseradish peroxidase (HRP). Tetramethylbenzidine (TMB) serves as the substrate. Assays were performed according to manufacturer's protocol. Data were analyzed with Softmax Pro and GraphPad Prism softwares.

All Sera and Plasma were obtained from BioIVT (Westbury, NY).

Human IL-22 Binding Protein (IL22-BP) was obtained from R & D Systems (Minneapolis, MN).

REFERENCES

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