VeriKine-HS™ Human IL-22 ELISA Kit (Cat. No. 41701-1)
Technical Data Sheet

Assay Range: 0.78 - 50 pg/ml
Compatibility: Serum, Plasma, Tissue Culture Media (TCM)
Assay Length: 4 hr

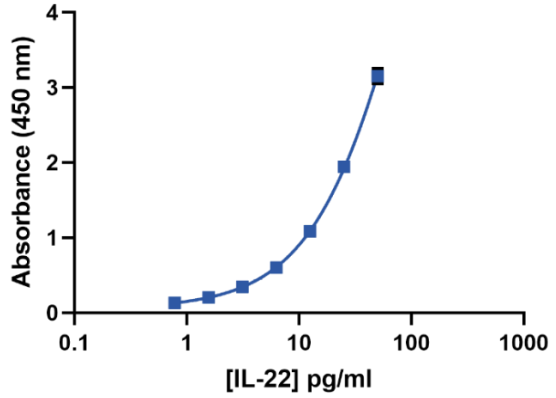
INTRODUCTION

Interleukin-22 (IL-22) is a member of the IL-10 family of cytokines[1]. Other names include IL-TIF and Zcyto18. 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 stromal 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, Rheumatoid Arthritis and Psoriasis[3]. IL-22 is often considered a hallmark of IL-17 driven immune responses[4].

PBL's IL-22 ELISA is intended for quantification of IL-22 in Tissue Culture Media (TCM), and complex matrices such as serum and plasma. The high affinity form of the IL-22 binding protein (IL22-BP) does inhibit this assay which suggests the ELISA may measure *free* IL-22 in samples.

Serum samples from 24 healthy donors and plasma samples from 7 healthy donors were all quantifiable and as such this assay may provide basal level measurement.

Figure 1. Typical Standard Curve in Standard Diluent Human IL-22 standard curves were prepared in Standard Diluent (SD) and assayed in triplicate over nine runs. The figure below shows the dose response and absorbance values. Error bars indicate the standard deviation.



Specifications This kit quantitates human IL-22 in serum, plasma and TCM using a sandwich immunoassay. The kit is based on an ELISA with biotinylated-detection antibody and streptavidin-conjugated horseradish peroxidase (HRP). Tetramethyl-benzidine (TMB) is the substrate.

Specificity Human IL-22. Limited cross-reactivity (< 1% non-specific detection) observed with monkey IL-22 (0.02% and 0.03% from two different runs). No cross-reactivity detected with human IL-10, IL-17, IL-19, IL-20, IL-24, IL-26/AK155, IFN-β 1a, IFN-γ, IFN-ω; cynomolgus IFN-α 2; cynomolgus/rhesus IFN-α; mouse IL-22, IFN-α A, IFN-β, IFN-γ; rat IL-22, IFN-α 1, IFN-α 14, IFN-β, IFN-γ; bovine IFN-Tau 2 or pig IFN-α.

PRECISION

Figure 2. Intra-Assay Precision 18 replicates of four Normal Human Serum (NHS) samples with different endogenous levels were tested on a single plate.

	Intra-Assay Precision			
Sample	**1**	**2**	**3**	**4**
n	18	18	18	18
Mean (pg/ml)	26.68	6.42	2.86	4.52
Standard Deviation	1.38	0.62	0.16	0.18
CV (%)	5.2	9.7	5.5	4.1

Figure 3. Inter-Assay Precision Five independent assays testing five NHS samples with different endogenous levels were run by the same operator.

	Inter-Assay Precision				
Sample	**1**	**2**	**3**	**4**	**5**
n	5	5	5	5	5
Mean (pg/ml)	28.05	6.73	3.13	4.80	5.79
Standard Deviation	3.62	0.68	0.17	0.27	0.54
CV (%)	12.9	10.1	5.3	5.7	9.3

Figure 4. Intermediate Precision Five NHS samples with different endogenous levels were tested over 27 assays by 7 operators using 3 lots of components.

	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 (%)	13.6	16.0	12.3	11.8	14.6

References:

1. Dumoutier, L., *et al*. 2000. Genes & Immunity. 1(8): 488-94.
2. Dudakov J., Hanash A., van den Brink, M. 2015. Annual Review of Immunology. 33: 747-785.
3. Eyerich, K., Dimartino, V., Cavani, A. 2017. European Journal of Immunology. 47(4): 607-614.
4. Eyerich, S., *et al*. 2017. Allergologie Select. 1(1): 71-76.

SPIKE RECOVERY

Figure 5. Spike Recovery Human IL-22 was spiked into two TCM, six Disodium-EDTA plasma and five NHS samples at three known concentrations (100, 25, and 5 pg/ml). Data was quantified in three runs.

TCM			
Spike Sample	**1**	**2**	**3**
Target Conc. (pg/ml)	100	25	5
Mean Recovery (%)	101.9	93.9	90.8
Range (%)	100.7-103.0	90.3-97.5	90.2-91.4
Disodium-EDTA Plasma			
Spike Sample	**1**	**2**	**3**
Target Conc. (pg/ml)	100	25	5
Mean Recovery (%)	97.3	88.7	83.9
Range (%)	87.6-102.6	82.0-93.3	76.1-92.5
Serum			
Spike Sample	**1**	**2**	**3**
Target Conc. (pg/ml)	100	25	5
Mean Recovery (%)	90.9	87.1	84.8
Range (%)	83.3-100.5	80.0-98.9	74.6-94.5

PERFORMANCE CHARACTERIZATION

Figures 6 & 7. Matrix Compliance Human IL-22 standard curves were prepared in NHS, Standard Diluent (SD) and TCM. Figure 6 shows dose response curves and absorbance values in various matrices. The NHS sample contains endogenous human IL-22. Figure 7 shows the percent recoveries of Human IL-22 standard at different concentrations for the various matrices. The SD curve was used as reference for calculating the percent recovery of both the NHS and TCM curves. Endogenous levels of human IL-22 were subtracted to calculate the corrected backfit concentrations of the standard in Figure 7. Standard curves were assayed in triplicate. Both figures show the mean of nine runs and error bars indicate the standard deviation.

Figure 6. Representative Standard Curves in Various Matrices

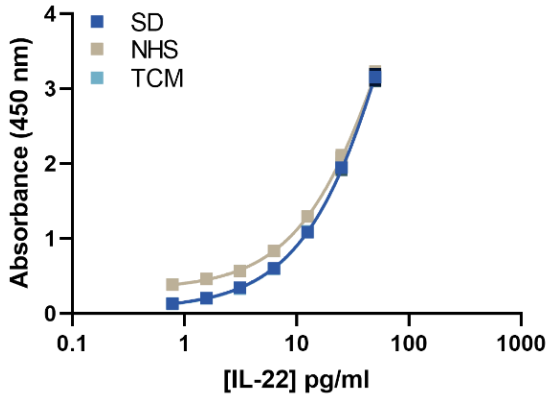


Figure 7. Percent Recovery of IL-22 in Various Matrices

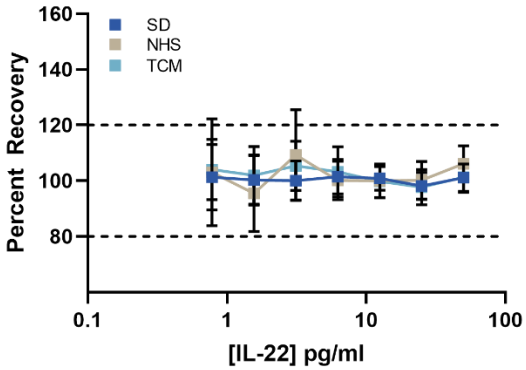


Figure 8. Parallelism of Normal Serum Three NHS samples with high levels of endogenous IL-22 were assayed in duplicate to assess reliable quantification after dilution within the standard curve range. Endogenous samples were diluted in Standard Diluent down to a 16-fold dilution. All samples fell within 100 ± 20% of the neat value. Error bars indicate the standard deviation.

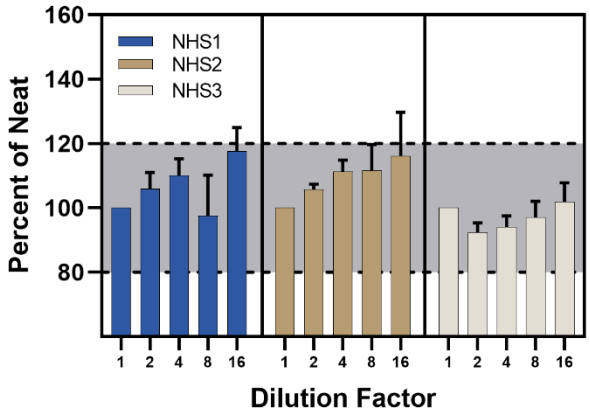
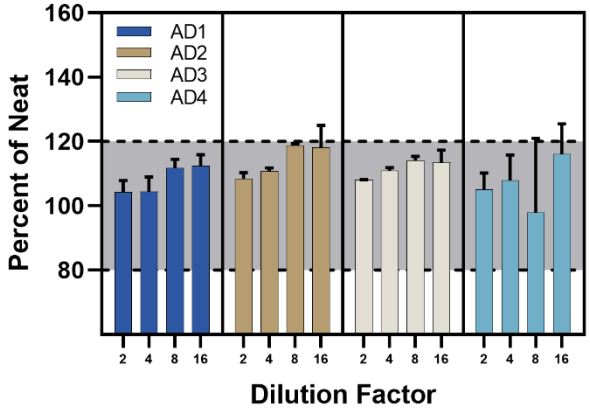


Figure 9. Parallelism of Disease State Serum Four *Atopic Dermatitis* (AD) serum samples with high levels of endogenous IL-22 were assayed in duplicate to assess reliable quantification after dilution within the standard curve range. Samples were diluted in Standard Diluent (SD) down to a 16-fold dilution. All samples fell within 100 ± 20% of the neat value. Error bars indicate the standard deviation.



VeriKine-HS™ Human IL-22 ELISA Kit (Cat. No. 41701-1)
Technical Data Sheet

Assay Range: 0.78 - 50 pg/ml
Compatibility: Serum, Plasma, Tissue Culture Media (TCM)
Assay Length: 4 hr

Figures 10, 11 & 12. Linearity of NHS, Normal Disodium-EDTA Plasma and Normal Sodium-Heparin Plasma Four NHS, five Disodium-EDTA plasma and five Sodium-Heparin samples from healthy volunteers with low endogenous levels of IL-22 were spiked with a known concentration of IL-22. Samples were diluted two-fold in Standard Diluent (SD) 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 10. Linearity of NHS

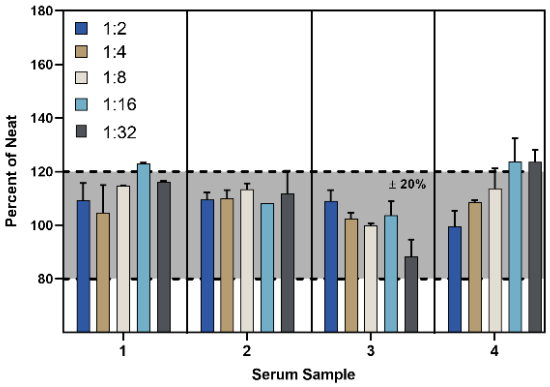


Figure 11. Linearity of Disodium-EDTA Plasma

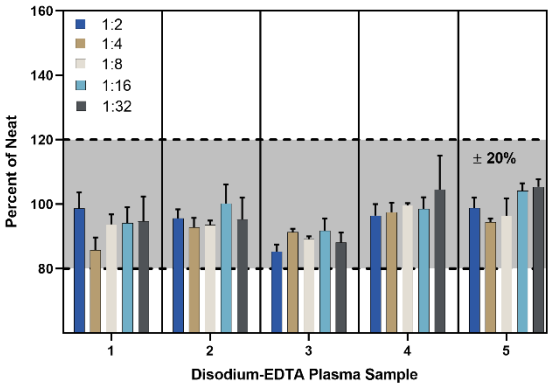
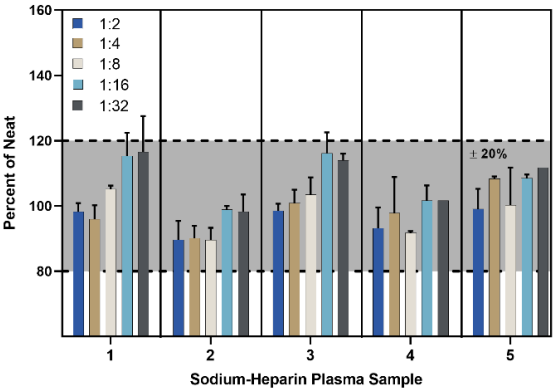


Figure 12. Linearity of Na-Heparin Plasma



PERFORMANCE COMPARISON

Summary PBL's IL-22 ELISA was internally characterized against Competitor A's IL-22 ELISA. PBL exhibited higher sensitivity than Competitor A. Note that PBL's standard is human cell-expressed (HEK293) whereas Competitor A's standard is *E.coli*-expressed.

Figure 13. Representative Standard Curves PBL's and Competitor A's standards were assayed on the 41701 PBL ELISA. Figure 13 shows the dose response curves and absorbance values in Standard Diluent (SD). Competitor A's standard exhibits a lower dose response compared to PBL's standard, as well as a lower percent recovery, which is less than 80% of the expected value. Competitor A's standard curve demonstrated less sensitivity than PBL's IL-22 ELISA.

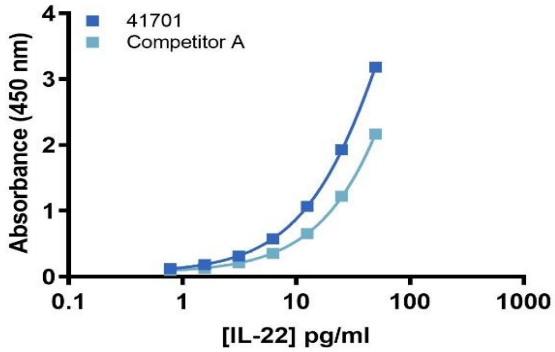
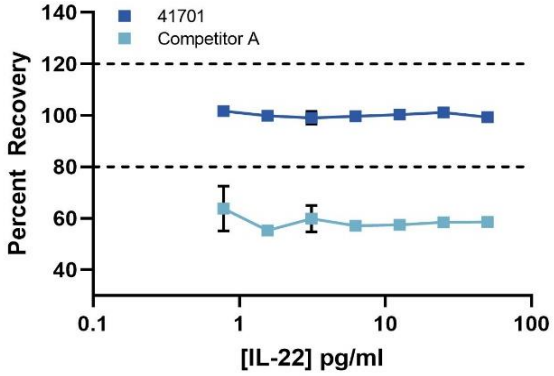


Figure 14. Comparison of Percent Recovery Curves The figure below shows the recovery of the individual IL-22 standards at various concentrations. Competitor A's standard was backfitted against PBL human cell-expressed standard, which was used to interpolate the concentrations of IL-22 and calculate the percent recovery of the curves from the expected value. PBL's ELISA exhibited higher percent recovery than Competitor A.



Figures 15 & 16. Representative Standard Curves (IL22-BP) To test whether soluble IL-22 Binding Protein (IL22-BP) interferes with the detection of IL-22 in Competitor A's or PBL's ELISA, 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 or Calibrator Diluent. Incubation with 10 ng/ml and 1 ng/ml of active IL22-BP interfered with detection of IL-22 in both ELISAs. Incubation with 10 pg/ml and 100 pg/ml did not interfere with detection in either kit. It can be concluded that both ELISAs detect free IL-22. Data was generated from a single run.

Figure 15. PBL's IL-22 ELISA (41701) Standard Curve in the Presence of IL22-BP

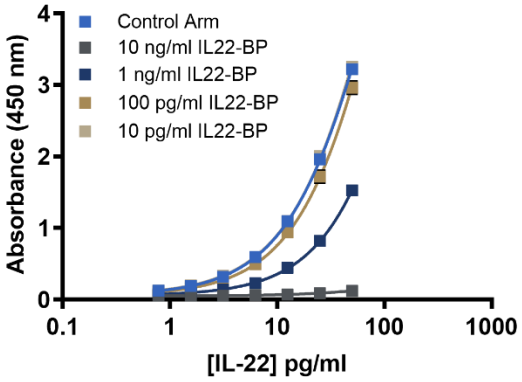
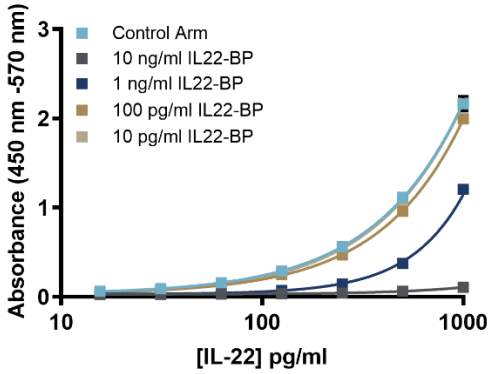


Figure 16. Competitor A's Standard Curve in the Presence of IL22-BP



Figures 17 & 18. Recovery Comparison in the Presence of IL-22BP The following figures show the recovery of IL-22 standards at various concentrations when pre-incubated in both the absence and presence of active 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. Error bars indicate the standard deviation.

Figure 17. PBL's IL-22 ELISA (41701) Recovery in the Presence of IL22-BP

High recovery rate in the presence of up to 100 pg/ml

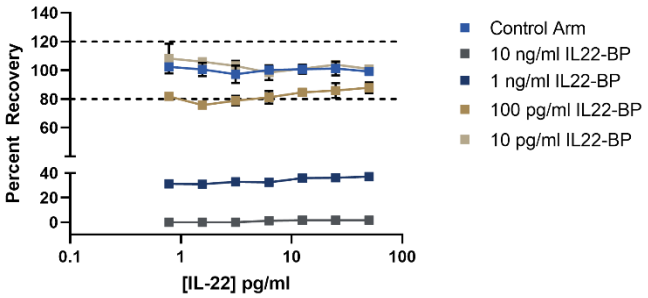
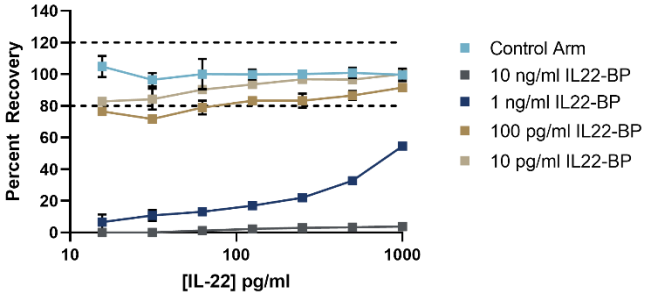


Figure 18. Competitor A Recovery in the Presence of IL22-BP



Figures 19 & 20. Healthy Donor Readability Comparison 31 samples from healthy volunteers were assayed on PBL's and Competitor A's kit. 24 NHS samples and 7 human Sodium-Citrate plasma samples were assayed in duplicate on each respective kit. PBL's has the lowest sensitivity (0.78 pg/ml) in the market and can detect very low endogenous levels in healthy samples. Competitor A could only detect 2 out of the 24 serum samples while PBL's detected 23 out of the 24 samples assayed. Competitor A did not detect any IL-22 in the seven plasma samples while PBL's detected very low endogenous levels in all **7** samples.

Figure 19. Healthy Donor Readability on PBL's ELISA

Sample Type	Mean of Detectable (pg/ml)	Range (pg/ml)	% Detectable	% Non-Detectable	%CV
NHS	1.84	0.80 – 5.43	96 23 out of 24	4	5.6
Sodium-Citrate Plasma	2.54	1.06 – 5.92	100 7 out of 7	0	4.5

Figure 20. Healthy Donor Readability on Competitor A's ELISA

Sample Type	Mean of Detectable (pg/ml)	Range (pg/ml)	% Detectable	% Non-Detectable	%CV
NHS	20.43	16.26 – 24.60	8 2 out of 24	92	2.8
Sodium-Citrate Plasma	ND	ND	0	100	ND

ND: Not Detectable

VeriKine-HS™ Human IL-22 ELISA Kit (Cat. No. 41701-1)
Technical Data Sheet

Assay Range: 0.78 - 50 pg/ml
Compatibility: Serum, Plasma, Tissue Culture Media (TCM)
Assay Length: 4 hr

Figures 21 & 22. Disease State Readability Comparison 40 serum samples from various diseased volunteers were assayed on PBL's and Competitor A's ELISAs. PBL's detected low and high endogenous levels in all 40 disease state samples. Competitor A's detected 5/10 *Atopic Dermatitis (AD)*, 1/10 *Psoriasis,* 0/10 *Rheumatoid Arthritis (RA)* and 0/10 *Systemic Lupus Erythematosus (SLE)* serum samples.

Figure 21. Disease State Readability on PBL's ELISA

Sample Type	Mean of Detectable (pg/ml)	Range (pg/ml)	% Detectable	% Non-Detectable	%CV
AD	14.77	2.30 – 32.16	100	0	2.1
Psoriasis	5.13	1.25 – 25.99	100	0	3.8
RA	5.58	1.98 – 9.47	100	0	3.7
SLE	3.13	1.55 – 9.22	100	0	4.7

Figure 22. Disease State Readability on Competitor A's ELISA

Sample Type	Mean of Detectable (pg/ml)	Range (pg/ml)	% Detectable	% Non-Detectable	%CV
AD	32.42	14.28 – 73.07	60	40	5.0
Psoriasis	33.09	N/A	10	90	2.9
RA	ND	ND	0	100	ND
SLE	ND	ND	0	100	ND

ND: Not Detectable

Visit the product page on PBL's website
(https://pblassaysci.com)
to view the one-page CoA/Protocol and
further information on this product (including references).