# Performance Characterization of a High Sensitivity Human IL-22 ELISA Kit in Healthy Human Donor Serum & Plasma Samples and Tissue Culture Media

## (Assay Range 0.78 – 50 pg/ml)

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## INTRODUCTION

Interleukin-22 (IL-22) is a member of the IL-10 family of cytokines[1]. 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 activation plays a role in the initiation and regulation of nonspecific immune response. It has both protective effects, such as hepatocyte & epithelial cell survival and pro-inflammatory effects. It is also implicated in Autoimmune diseases (AD) 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].

PBL's IL-22 Elisa kit is validated to quantify IL-22 levels in complex matrices such as Healthy Human Donor Serum & Plasma and Tissue Culture Media (TCM). The high affinity form of the IL-22 binding protein (IL22-BP) does inhibit this assay, which suggests that this ELISA may measure *free* IL-22 in samples.

## Assay Format

PBL's *VeriKine High Sensitivity Human IL-22 ELISA (Catalog No. 41701-1)* has a 96 well plate coated with IL-22 capture antibody. The detection antibody is a biotinylated secondary antibody, followed by streptavidin conjugated to horseradish peroxidase (HRP) step where TMB (Tetramethylbenzidine) serves as a substrate.
The total length of the assay is 4 hours, with 0.78 - 50 pg/ml calibration range and <1 pg/ml LOQ. It accurately quantitates sub picogram levels of endogenous IL-22 in healthy human donor serum (HDS) & plasma (HDP) samples. IL-22 standard has been expressed in Human Embryonic Kidney (HEK-293) cells. Detailed studies were performed to assess the accuracy, sensitivity and robustness of this kit.

## RESULTS

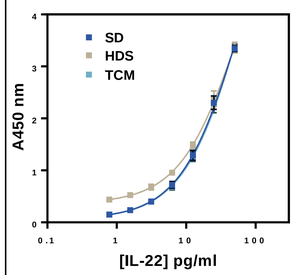


**Fig 1. Typical Standard Curves in SD, HDS & TCM** are shown. Standard diluent (SD) and TCM curves overlap, but HDS curve is shifted. The shift is due to the endogenous IL-22 (quantified separately), in the HDS sample which results in a vertical A-450 nm displacement through-out the HDS curve. Data shown is a mean of nine runs, run in triplicate. IL-22 recoveries for all matrices ranged 100% ± 20% (data not shown).

| Spike Recovery | | Target Conc. (pg/ml) | Mean Recovery (%) | Range (%) |
|---|---|---|---|---|
| High Spike | TCM | 30 | 101.9 | 100.7- 103.0 |
| | Disodium-EDTA Plasma | 30 | 97.3 | 87.6 - 102.6 |
| | Serum | 30 | 90.9 | 83.3 - 100.5 |
| Medium Spike | TCM | 10 | 93.9 | 90.3 - 97.5 |
| | Disodium-EDTA Plasma | 10 | 88.7 | 82.0 - 93.3 |
| | Serum | 10 | 87.1 | 80.0 - 98.9 |
| Low Spike | TCM | 3 | 90.8 | 90.2 - 91.4 |
| | Disodium-EDTA Plasma | 3 | 83.9 | 76.1 - 92.5 |
| | Serum | 3 | 84.8 | 74.6 - 94.5 |

**Fig 2. Spike Recovery** TCM (n=2), Plasma (n=6) & Serum (n=5) samples were spiked with IL-22 standard at High, Medium & Low target concentrations. The table shows the mean IL-22 recovery for each sample matrix (83.9 – 101.9%).

| PRECISION | Serum # | 1 | 2 | 3 | 4 | 5 |
|---|---|---|---|---|---|---|
| Intra-Assay Precision (18 replicates, 4 NHS samples, run on a single plate) | Mean (pg/ml) | 26.68 | 6.42 | 2.86 | 4.52 | n/a |
| | Std. Dev. | 1.38 | 0.62 | 0.16 | 0.18 | n/a |
| | CV (%) | 5.2 | 9.7 | 5.5 | 4.1 | n/a |
| Inter-Assay Precision (5 Elisa runs, 1 operator, 1 kit lot) | Mean (pg/ml) | 28.05 | 6.73 | 3.13 | 4.8 | 5.79 |
| | Std. Dev. | 3.62 | 0.68 | 0.17 | 0.27 | 0.54 |
| | CV (%) | 12.9 | 10.1 | 5.3 | 5.7 | 9.3 |
| Intermediate-Precision (27 Elisa runs, 7 operators, 3 kit lots) | Mean (pg/ml) | 31.93 | 7.76 | 3.43 | 5.3 | 6.45 |
| | Std. Dev. | 4.34 | 1.24 | 0.42 | 0.62 | 0.94 |
| | CV (%) | 13.6 | 16 | 12.3 | 11.8 | 14.6 |

**Fig 3. Precision studies.** Intra-assay, Inter-assay & Intermediate precision for PBL's ELISA kit is summarized in the table above. Single serums or Serum Pool with varying endogenous IL-22 levels were used for this study.

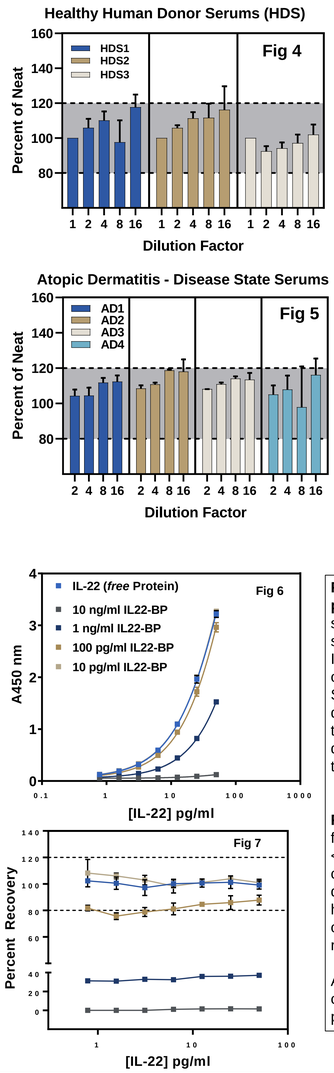


**Fig 4, 5. Endogenous Dilution Linearity** on three HDS samples with high endogenous IL-22 and four Atopic Dermatitis (AD) diseased state sera (~18-28 pg/ml) is shown. All samples were diluted 2-fold in the standard diluent and percent recoveries calculated after adjusting for the fold dilution.

Recovery for all samples was recorded largely within 100 ± 20% of the neat value.

Error bars indicate the standard deviation between the replicates.




**Fig 6. IL-22 BP inhibits the *free* protein IL-22 standard curve -** standard curves were prepared in standard diluent (SD) with & without IL22-BP. Graph shows that 1-10 ng/ml of soluble IL-22BP added to the IL-22 SD curve, interferes with *free* IL-22 detection. But, at <100 pg/ml IL22-BP there is no interference in IL-22 detection. Thus, it can be concluded that PBL ELISA detects *free* IL-22.

**Fig 7. Standard Curve Recovery** from Fig 6 is shown. IL-22 BP at <100pg/ml added to the IL-22 SD curve recovered IL-22 largely in the desirable range 100% ± 20%. But at higher IL-22 BP levels (1-10 ng/ml), only <40% of *free* IL-22 was recovered.

All curves were backfitted to the control IL-22 standard curve (*free* protein) in SD without any IL-22BP.

| Comparison with Other Suppliers | | |
|---|---|---|
| **Fig 8** | **PBL** | **Supplier A** |
| Assay range (pg/ml) | 0.78 - 50 | 15.6 - 1000 |
| Assay Sensitivity (pg/ml) | <1 | 5.8 |
| IL-22 Standard Cell expression | HEK-293 | *E. Coli* |
| IL-22 Standard Protein form | *Free* IL-22 | *Free* IL-22 |
| IL-22 inhibition by IL-22BP | Yes | Yes |
| **Healthy Human Donor Sample Readability** | | |
| Serum (n=24) | 96% | 8% |
| Plasma (Sodium Citrate) (n=7) | 100% | 0% |
| **Diseased Sample Readability** | | |
| Atopic Dermatitis (n=10) | 100% | 60% |
| Psoriasis (n=10) | 100% | 10% |
| Rheumatoid Arthritis (n=10) | 100% | 0% |
| Systemic Lupus Erythematosus (n=10) | 100% | 0% |

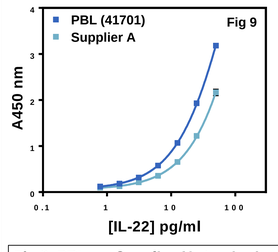


**Fig 9. PBL vs. Supplier A' standard curves** IL-22 standards of PBL and Supplier A' were run on the PBL kit (41701-1). Graph shows the absorbance values and dose response curves in standard diluent. Supplier A's standard exhibits a lower dose response as compared to the PBL's standard.

**Fig 10. Percent Recovery** of IL-22 from Fig 9 is shown. Supplier A's *E Coli* IL-22 standard was backfitted against PBL's HEK IL-22 standard. PBL's standard shows a desirable recovery (100% ± 20%) while Supplier 'A' had a recovery <80%.

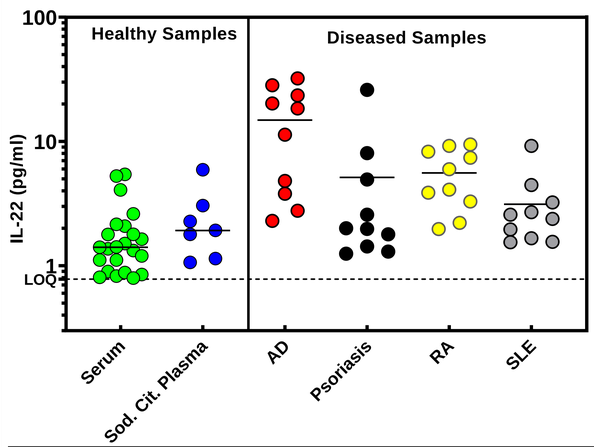


**Fig 11. Healthy & Diseased Sample Readability in PBL's ELISA (41701-1)** Low IL-22 levels in Healthy Donor Serum/Plasma samples were reproducibly quantified in 1-10 pg/ml range. LOQ for PBL's kit = 0.78 pg/ml. Similarly, a few relevant Diseased state samples were quantified as shown & also summarized in Fig 8 table, such as - Atopic Dermatitis (AD), Psoriasis, Rheumatoid Arthritis (RA) and Systemic Lupus Erythematosus (SLE). All samples were tested in duplicate.

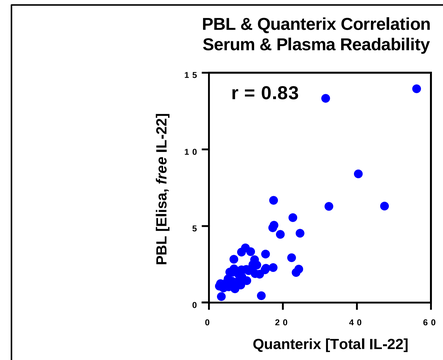


**Fig 12. Healthy donor sample readability correlation r = 0.83** between PBL & 'Quanterix' is shown. 'Quanterix' uses SIMOA, a bead-based technology and is a pioneer in business with serum & plasma detectability in '*femtogram*' range. Two different technologies, but similar results between the two suppliers validates the accuracy of PBL's Elisa kit to measure *free* IL-22 in the similar range as 'Quanterix' (Total IL-22 = free + receptor bound). IL-22 in healthy human donor samples is in 1-10 pg/ml range.

## SUMMARY

PBL's *VeriKine High Sensitivity Human IL-22 ELISA (Catalog No. 41701-1)* has an IL-22 standard expressed in HEK-293 cells. The high affinity form of IL-22 binding protein (IL22-BP) inhibits the measurement of *free* IL-22 protein at >100 pg/ml IL-22BP concentrations, suggesting that our ELISA may measure *free* IL-22. PBL's kit accurately quantitates IL-22 in Healthy Human Donor Serum & Plasma and Tissue Culture Media.

**Key Takeaways:**

1. PBL's IL-22 ELISA calibration range is 0.78 - 50 pg/ml and <1 pg/ml LOQ. IL-22 standard of this kit is expressed in HEK-293 cells.
2. Endogenous IL-22 in 100% healthy human donor serum & plasma samples was reliably and reproducibly quantitated in the expected 1-10 pg/ml range (Fig 11, 12). Similarly, 100% of the relevant Diseased state samples were quantifiable in PBL's kit (Fig 8, 11).
3. Excellent correlation between 'PBL' and 'Quanterix' (r = 0.83) further validated, accurate IL-22 measurements by the PBL's kit. Two suppliers, two different technologies, yet similar results (Fig 12).
4. Endogenous IL-22 dilution linearity (Fig 4, 5) and Precision studies (Fig 3) further confirmed the performance of this kit. Rigorous validation of this kit was done to test for sensitivity, accuracy and reproducibility.
5. Spiked IL-22 recovery in complex matrices such as healthy human donor serum & plasma and TCM samples was also recorded in the desirable 100 ± 20% range (Fig 2).

## REFERENCES

1. Dumoutier, L., *et al.* 2000. Genes & Immunity. 1(8): 488-94.
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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.

## FURTHER INFORMATION

Find resource link here for additional performance studies.
https://www.pblassaysci.com/assay-kits/elisas/human-interleukin-elisas/human-il-22-elisa