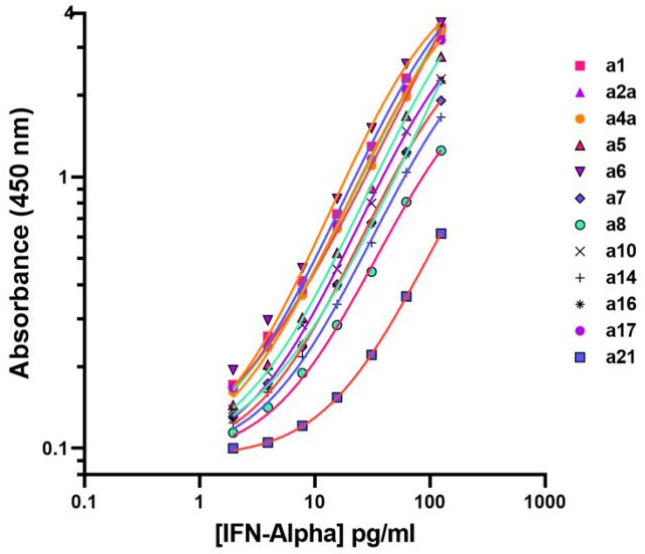
VeriKine-HS™ Human Interferon Alpha All Subtype ELISA Kit
Technical Data Sheet

Assay Range: 1.95 - 125 pg/ml
Compatibility: Serum, Tissue Culture Media (TCM)
Assay Length: 22 hr 30 min

INTRODUCTION

In humans, IFN-Alpha consists of a group of proteins that are greater than 85% homologous by amino acid sequence. Numerous individual human IFN-Alpha subtypes have been identified; many display different properties. It remains unclear why there are multiple IFN-Alpha subtypes. A variety of studies suggested they possess overlapping but also unique sets of biological activities.

Figure 1. Representative Human IFN-Alpha Subtype Standard Curves in Healthy Donor Serum (HDS)



Specifications This kit quantitates human IFN-Alpha in human sera, plasma, and tissue culture media (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. The assay is based on the international reference standard for human IFN-Alpha provided by the National Institutes of Health.

Specificity Human IFN-Alpha. Cross reacts with Cynomolgus/Rhesus IFN-Alpha. No cross reactivity detected with human IFN-Beta, IFN-Gamma or IFN-Omega; mouse or rat IFN-Alpha, IFN-Beta or IFN-Lambda; or bovine IFN-Tau.

PRECISION

Figure 2. Intra-Assay and Inter-Assay Precision To test precision within and between assays, Standard Diluent (SD), serum pool, and TCM matrix standard curves were assayed in triplicate. A total of 27 assays were conducted across three different batches of ELISA kits.

	Intra- and Inter-Assay Precision		
Matrix Type	**SD**	**Serum Pool**	**TCM**
n	27	27	27
Intra-Assay CV (%)	2.2	1.9	2.2
Inter-Assay CV (%)	8.5	7.5	7.4

SPIKE RECOVERY

Figure 3. Spike Recovery Human IFN-Alpha was spiked into Healthy Donor Serum (HDS) at three known concentrations (3, 9, and 90 pg/ml). 18 independent assays were run among two batches of ELISA kits.

	Serum Spike Recovery		
Spike Sample	**1**	**2**	**3**
Target Conc. (pg/ml)	3	9	90
Average Recovery (%)	120.5	102.7	95.4
Range (%)	99.8-146.3	85.8-123.5	85.6-101.0

PERFORMANCE CHARACTERIZATION

Figure 4. Lot-to-Lot Consistency Standard curves from 5 different lots were evaluated and compared to lot L-7010. Expired kit lots (denoted by italics and an asterisk (*)) were tested as part of an ongoing stability study. L-6527 and L-6473 were tested at 2 years post-expiration. IFN-Alpha was measurable with an accuracy of +/- 20% of the expected value. Error bars indicate the standard deviation.

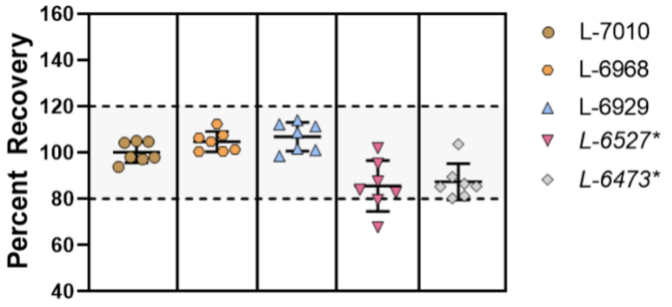


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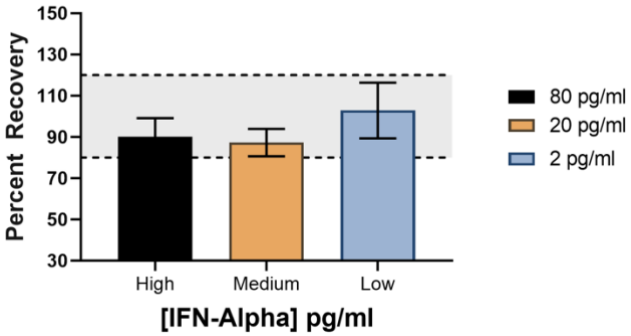
Figure 5. Spike Recovery of TCM Using Sendai Virus-Infected Cell Supernatant Myeloid lymphocyte cells (U937), cultured in RPMI medium supplemented with 10% FBS, were infected with Sendai virus and incubated for 24 hours, prior to collecting supernatant. The IFN-Alpha concentration of the supernatant was characterized and used to prepare three target spike levels diluted in Standard Diluent. High (80 pg/ml), medium (20 pg/ml), and low (2 pg/ml) spikes were assayed in quadruplicate over two independent runs. IFN-Alpha was measurable with an accuracy of +/- 20% of the expected value. Error bars indicate the standard deviation on the observed mean.



Figures 6, 7, 8 & 9. Linearity of Dilution Five HDS, five Disodium-EDTA plasma, five Sodium Citrate plasma, and four Sodium Heparin plasma samples from healthy donors with low background were spiked with a known concentration of IFN-Alpha (85 pg/ml). Samples were diluted two-fold in Standard Diluent to assess reliable quantification after dilution within the concentration range of the standard curve. Samples were diluted down to a 32-fold dilution, and endogenous levels were subtracted from dilution-corrected backfit concentrations. IFN-Alpha was detectable with an accuracy of +/- 20% of the neat (undiluted) calculated concentration.

Figure 6. Linearity of Healthy Donor Serum (HDS)

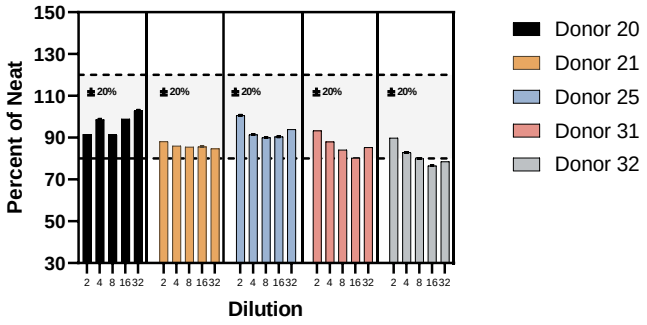


Figure 7. Linearity of Disodium-EDTA Plasma

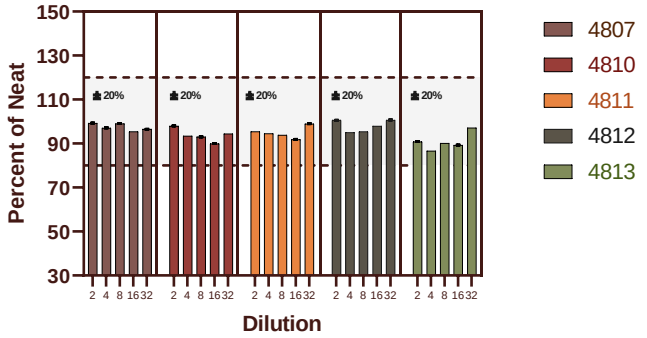


Figure 8. Linearity of Sodium Citrate Plasma

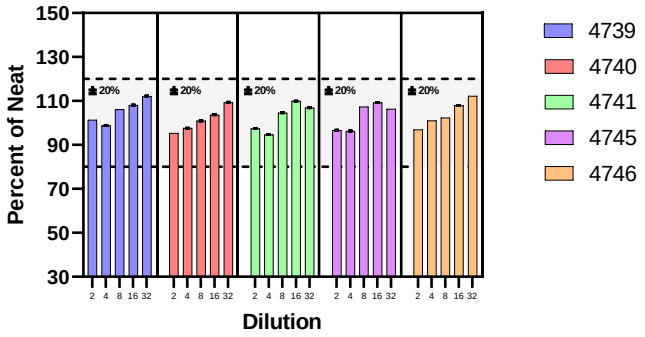


Figure 9. Linearity of Sodium Heparin Plasma

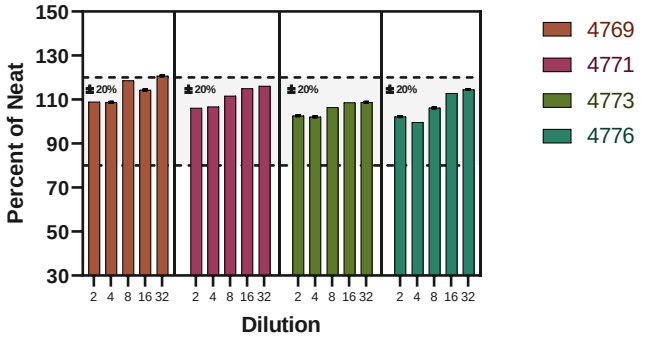


Figure 10. Measurement of Healthy and Influenza Infected Donors 64 serum samples from healthy donors and 38 serum samples from donors, clinically presenting Influenza, were assayed on PBL's ELISA. Samples were assayed neat (undiluted) or under a requisite dilution with Standard Diluent. Within the influenza cohort, 68.4% exhibited readable levels of IFN-Alpha above the ELISA LOD. All healthy donors assayed did not present any detectable level of IFN-Alpha.

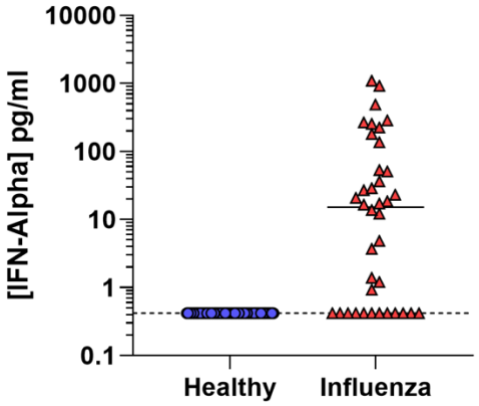
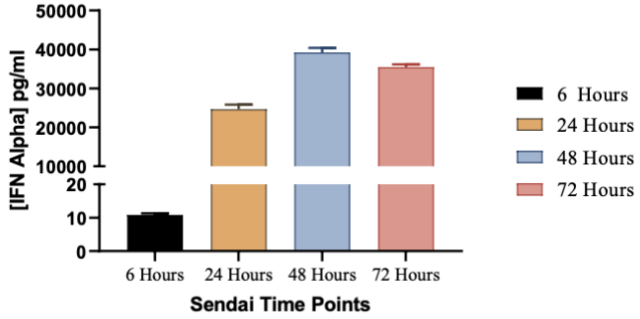
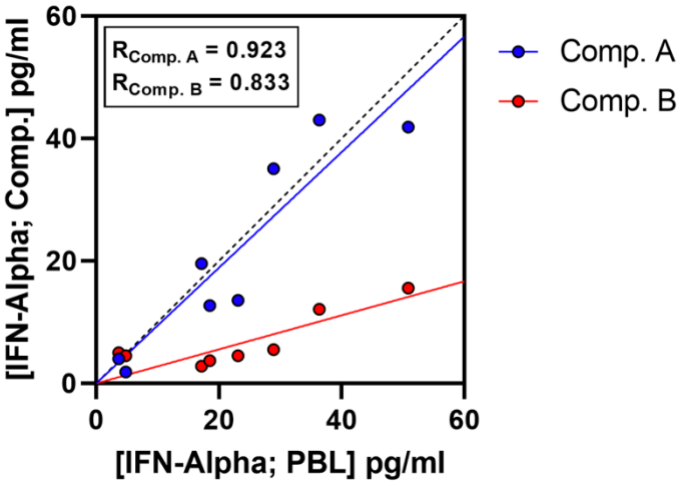


Figure 11. Levels of IFN-Alpha Quantified in Sendai Virus Time Course U937 cells were infected with 1.5 HA units of Sendai Virus. Supernatants were collected at 6, 24, 48, and 72 hours post-infection. Anticipating high IFN-Alpha levels, samples were diluted 1:500 in Standard Diluent prior to the start of the assay with the exception of the 6 hour time point. Uninfected controls were assayed alongside infected samples and added neat (undiluted) onto plate. All uninfected samples (negative controls) outputted at similar OD values to the plate blank of 0.08, indicating no quantifiable levels of IFN-Alpha. The standard curve and samples were assayed in triplicate, and sample concentrations were interpolated from assay calibration. Error bars indicate the standard deviation.

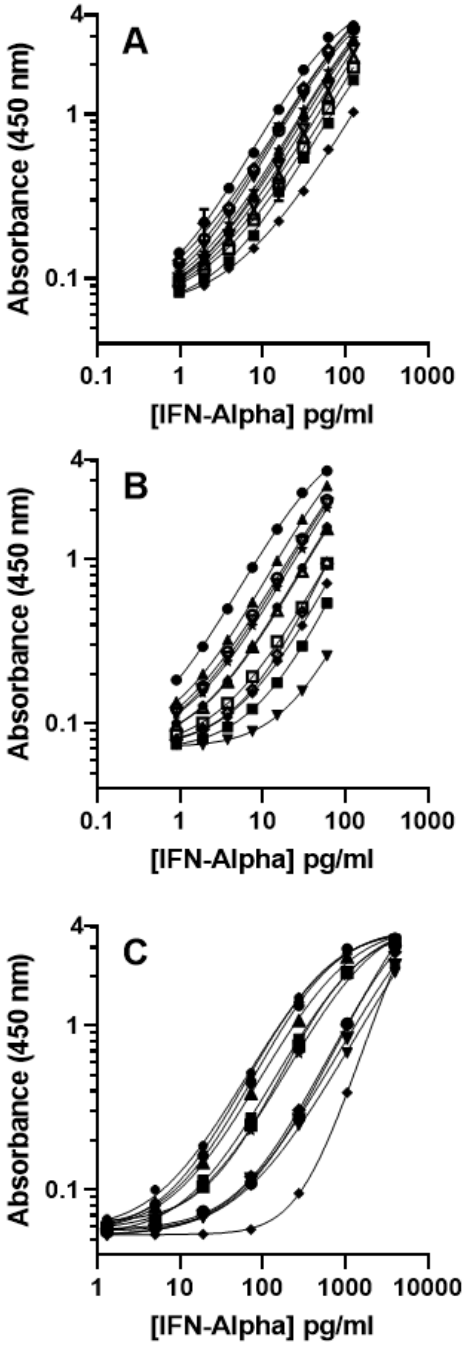


PERFORMANCE COMPARISON

Figure 12. Correlation between Detected Levels of IFN-Alpha Among PBL's 41115 ELISA and Competitor ELISAs Eight paired Influenza samples displaying readable levels of IFN-Alpha were assayed on two competitor kits to determine correlation against the values obtained on PBL's 41115 ELISA. Competitor A provides a correlation coefficient of 0.923 with a slope of 0.94. Competitor B ELISA provided a correlation coefficient of 0.833 with a slope of 0.28. These differences indicate that the breadth of subtyping detection and selectivity in IFN-Alpha interrogation may have different interpretations based on the technology applied within bench studies.



Figures 13, 14 & 15. Comparative Profile of all 12 Human IFN-Alpha subtypes on PBL's (A) and Two Commercial ELISAs (B, C)



Visit PBL's website (pblassaysci.com/documentation) for additional information including CoAs & Protocols