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

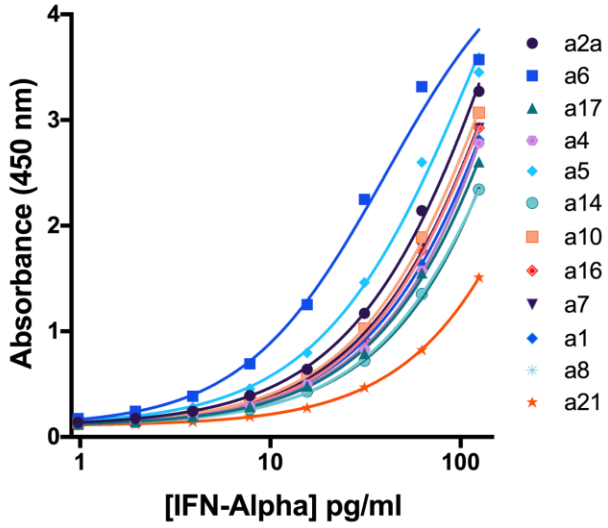
Assay Range: 1.95 - 125 pg/ml
Compatibility: Tissue Culture Media (TCM)
Assay Length: 20 hr 30 min – 24 hr 30 min

INTRODUCTION

In humans, IFN-Alpha refers to a family of proteins comprised of 12 highly homologous protein subtypes (greater than 85% by amino acid sequence), encoded by 13 genes, that exhibit pleiotropic biologic activities. Measuring the levels of the 12 different human IFN-Alpha subtypes is essential for understanding their triggers and subsequent effects on the immune system. The ability to measure all the subtypes may provide an indication of the total IFN-Alpha level.

Figures 1 & 2. Human IFN-Alpha All Subtype Detection & Typical Standard Curves of Human IFN-Alpha

Subtype	LLOQ
α1 (αD)	2.06
α2a (αA)	1.41
α4a (αM1)	2.03
α5 (αG)	1.20
α6 (αK)	0.82
α7 (αJ1)	1.93
α8 (αB2)	2.67
α10 (αC)	1.98
α14 (αH)	3.06
α16 (αWA)	2.39
α17 (αI)	2.26



Specifications This kit quantitates human IFN-Alpha in 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.

Specificity Human IFN-Alpha. Cross reacts with Cynomolgus/Rhesus IFN-Alpha (13%). No cross reactivity detected with human IFN-Beta, IFN-Gamma or IFN-Omega; mouse or rat IFN-Alpha, IFN-Beta or IFN-Gamma; pig IFN-Alpha; or bovine IFN-Tau.

PRECISION

Human IFN-Alpha was spiked into TCM at three known concentrations: 3, 15, and 90 pg/ml.

Figure 3. Intra-Assay CV To test precision within an assay, 20 replicates of each concentration were tested on a single plate.

	Intra-Assay Precision		
TC Media Spike Sample	**1**	**2**	**3**
n	20	20	20
Mean (pg/ml)	2.39	12.90	87.16
Standard Deviation	0.13	0.46	4.58
CV (%)	5.4	3.6	5.3

Figure 4. Inter-Assay CV To test precision between assays, 3 independent assays testing each spike concentration were run by the same operator. The results represent averaged data from 3 operators.

	Inter-Assay Precision		
TC Media Spike Sample	**1**	**2**	**3**
n	9	9	9
Mean (pg/ml)	3.29	15.13	93.69
Standard Deviation	0.16	0.83	6.07
CV (%)	4.9	5.5	6.5

Figure 5. Intermediate Precision To test intermediate precision, 25 assays were run by 10 operators using 3 lots of components.

	Intermediate Precision		
TC Media Spike Sample	**1**	**2**	**3**
n	25	25	25
Mean (pg/ml)	3.29	15.30	94.00
Standard Deviation	0.44	1.75	7.37
CV (%)	13.4	11.4	7.8

SPIKE RECOVERY

Figure 6. Spike Recovery Human IFN-Alpha was spiked into TCM at three known concentrations in 25 independent assays.

	Spike Recovery		
TC Media Spike Sample	**1**	**2**	**3**
n	25	25	25
Average Recovery (%)	109	102	104
Range	86-143	81-122	90-120

PERFORMANCE CHARACTERIZATION

Figure 7. Linearity Human IFN-Alpha was spiked into three tissue culture media (RPMI, DMEM and MEM) at 80 pg/ml and then serially diluted with Standard Diluent to assess assay linearity.

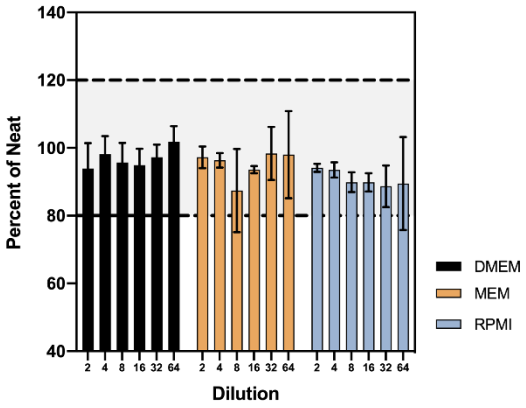


Figure 8. Parallelism of Endogenous Samples Five endogenous samples were tested on Cat No. 41135 and assayed in duplicate. Samples were serially diluted in TCM to the 32nd dilution, which still lies within standard curve range. Percent recoveries are 100±20% of the neat value. Cat. No. 41135 has acceptable parallelism. Error bars indicate standard deviation.

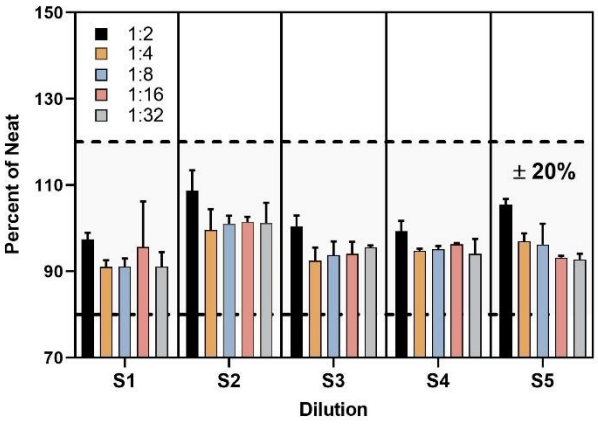
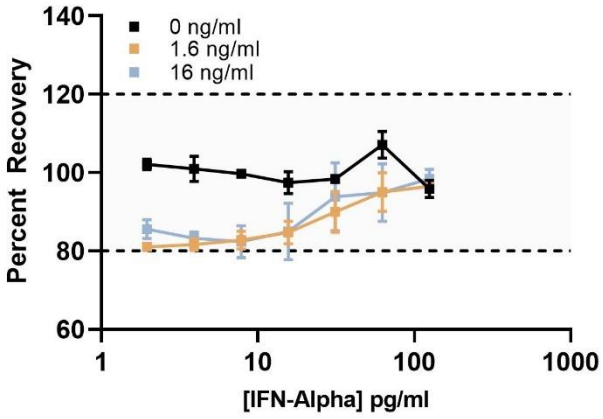


Figure 9. sIFNAR2 Receptor Interference To test whether the soluble IFN Alpha/Beta Receptor 2 (sIFNAR2) receptor interferes with the detection of IFN-Alpha in Cat No. 41135, two concentrations of standard (1.6 ng/ml and 16 ng/ml) were pre-incubated with sIFNAR2 for 1 hour at room temperature, prior to proceeding with the assay. Concentrations were interpolated from assay calibrator. Standard curves preincubated with sIFNAR2 were backfitted against standard curves without sIFNAR2. Error bars indicate standard deviation. (n=2)



COMPETITOR PERFORMANCE CHARACTERIZATION

Figure 10. Endogenous Levels of IFN-Alpha Quantified in Sendai Virus (A549) A549 cells were infected with 15 HA units of Sendai Virus (SeV). Supernatants were collected at five time points post infection (2, 6, 24, 48, and 72 hours). Samples were assayed in Cat. No. 41135, Competitor A's, and Competitor B's plates; there was no quantification on Competitor B's plate. 24, 48 and 72 hour time points were prediluted 1:10 in DMEM prior to start of assay for 41135 only. The standard curve and samples were assayed in duplicate. Sample concentrations were interpolated from assay calibrators from each respective vendor. Error bars indicate standard deviation.

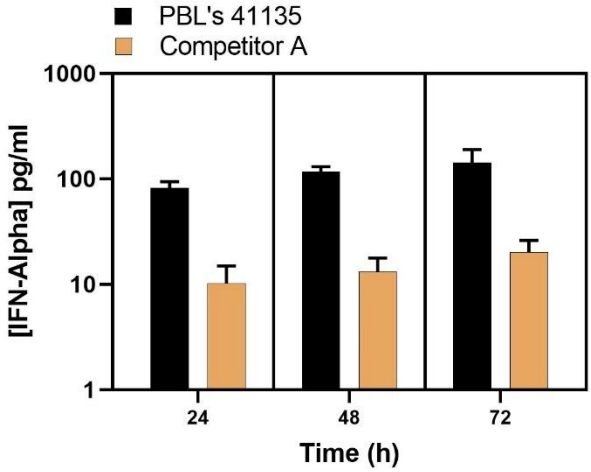
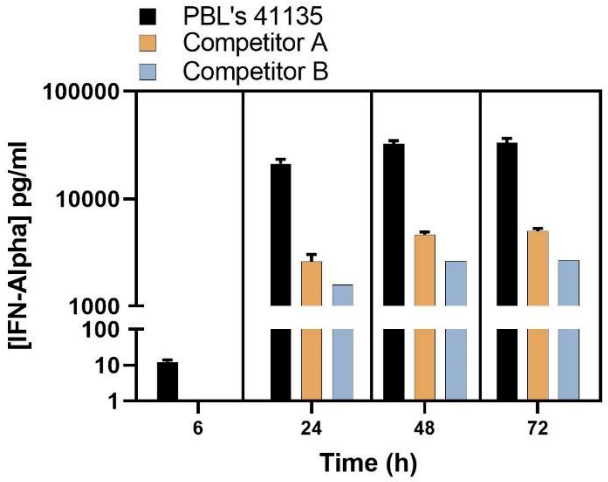


Figure 11. Endogenous Levels of IFN-Alpha Quantified in Sendai Virus (U937) U937 cells were infected with 1.5 HA units of Sendai Virus (SeV). Supernatants were collected at four timepoints post infection (6, 24, 48, and 72 hours). Samples were prediluted to 1:50 final dilution in RPMI prior to start of assay, except for 6 hour timepoint. The standard curve and samples were assayed in duplicate. Sample concentrations were interpolated from assay calibrators from each respective vendor. Error bars indicate standard deviation.



Visit the one-page COA & Protocol on PBL's website
(https://pblassaysci.com)
to view the full protocol,
including kit specifications and calculation of results.