VeriKine-HS Human Interferon Beta TCM ELISA Kit (Cat. No. 41435-1)

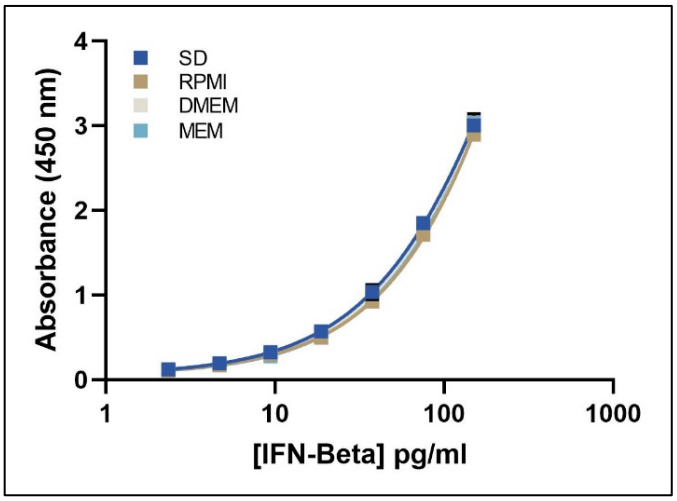
Technical Data Sheet

Assay Range: 2.34 - 150 pg/ml
Compatibility: Tissue Culture Media (TCM)
Assay Length: 3 hr

INTRODUCTION

In humans, Type I IFNs consist of multiple IFN-Alpha (α) genes and at least one IFN-Beta (β) gene in most vertebrates. IFN-Beta expression and secretion is primarily induced by signaling from pattern recognition receptors such as Toll-like (TLR) and RIG-I-like receptors (RLR). Overall, IFN-Beta is part of the first wave of cytokine response in cells. Pathogen infection can result in the activation of interferon regulatory factor 3 (IRF3) that functions *in trans* to activate IFN-Beta gene transcription.

Figure 1. Representative Human IFN-Beta Standard Curves in Various Matrices Human IFN-Beta standard curves were prepared in Tissue Culture Media (TCM) and Standard Diluent (SD). Three different TCM were used: RPMI, DMEM and MEM. TCM were supplemented with 10% FBS. Figure 1 shows the mean of two runs and error bars indicate the standard deviation.



Specifications This kit quantitates human IFN-Beta 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.

Specificity Human IFN-β. No cross-reactivity detected with human IFN-α, IFN-γ, IFN-ω or IL-6. No cross-reactivity with mouse IFN-α, mouse IFN-β or rat IFN-β.

Note The 41435 ELISA is not intended for use with human serum or plasma samples because of a significantly elevated risk of false positive results in these matrices.

PRECISION

Table 1. Intra and Inter-Assay CV To test precision within an assay (*intra*), and between assays (*inter*), independent assays testing IFN-Beta concentrations in Standard Diluent (SD) and Tissue Culture Media (TCM) were run by multiple operators. Values were assessed among various concentrations from the standard curve.

	Precision	
Matrix	**Standard Diluent**	**TCM**
n	7	18
Intra-Assay CV (%)	4.10	4.06
Inter-Assay CV (%)	2.33	3.16

SPIKE RECOVERY

Table 2. Spike Recovery Human IFN-Beta was spiked into TCM at three known concentrations in 2 independent assays.

	Spike Recovery		
TC Media Spike Sample	**1**	**2**	**3**
Target Conc. (pg/ml)	100	25	5
Mean Recovery (%)	101.1	95.7	91.4
Range (%)	97-106	90-102	85-96

PERFORMANCE CHARACTERIZATION

Figure 2. Matrix Compliance Human IFN-Beta standard curves were prepared in Tissue Culture Media (TCM) and Standard Diluent (SD). Three different TCM were used: RPMI, DMEM and MEM, each supplemented with 10% FBS. The figure below shows the recovery of Human IFN-Beta Standard at the different concentrations for the various matrices. The SD curve was used as reference for calculating the percent recovery of IFN-Beta in TCM from the expected value. The figure shows the means of two runs, and error bars indicate the standard deviation.

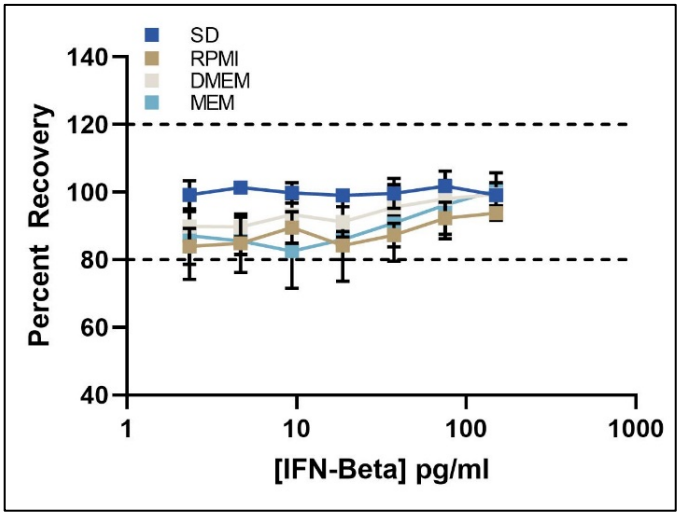
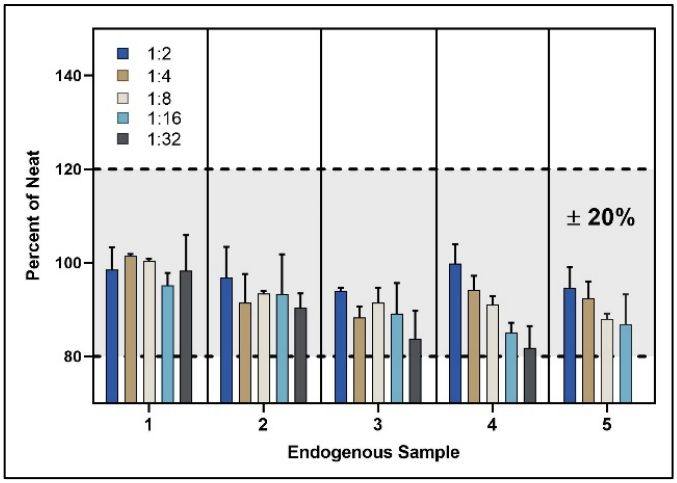


Figure 3. Parallelism of Endogenous Samples Five endogenous samples were tested and assayed in duplicate. Samples were serially diluted in TCM to the 32-fold dilution, which still lies within standard curve range. Percent recoveries are 100 ± 20% of neat value except for the 32nd dilution of Sample 5 (S5) which falls just beneath the bottom standard curve point of 2.34 pg/ml. Error bars indicate the standard deviation.



Knockdown of Endogenous Samples A knockdown experiment was conducted to test the specificity of the 41435 ELISA on apparent IFN-Beta within Sendai Virus (SeV)-induced HEK293 time course samples. Six endogenous samples with concentrations ranging from 495.7 to 1438.5 pg/ml were tested. Samples were preincubated overnight at 4°C with either 1 x PBS or equal volume of the unbiotinylated detection antibody or of the nonspecific irrelevant matched isotype control antibody. The samples were assayed on the plate the following day and kit protocol was followed. All samples incubated with the unbiotinylated detection antibody resulted in total knockdown of the signal, and OD values similar to blank OD values of 0.05. Samples incubated with the matched isotype control antibody did not inhibit the specific signal, and resulted in no greater than 20% deviation than control mean.

Figure 4. Sensitivity in Stimulated PBMC Samples PBMCs were isolated from a healthy individual's buffy coat and stimulated with 10 ug/ml of the TLR-7/8 agonist, R-848, to induce detectable levels of IFN-Beta in RPMI. Four densities of PBMCs were used (1E6, 2E6, 3E6 and 4E6 cells per ml), and supernatants were collected 24 and 48 hours post stimulation. The 1E6 PBMC sample falls below the LLOQ (Lower Limit of Quantitation) of 2.34 pg/ml.

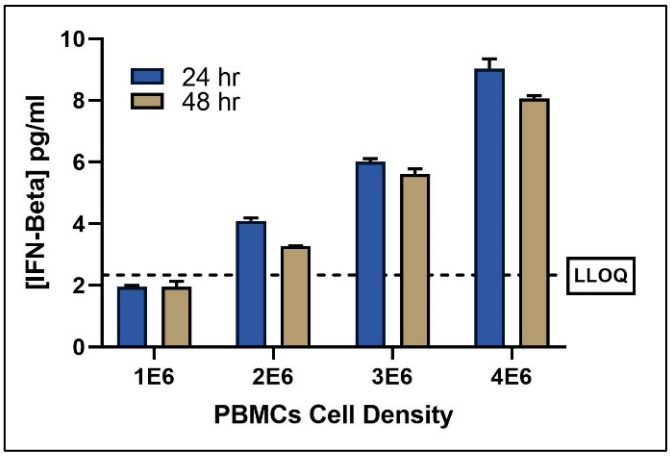
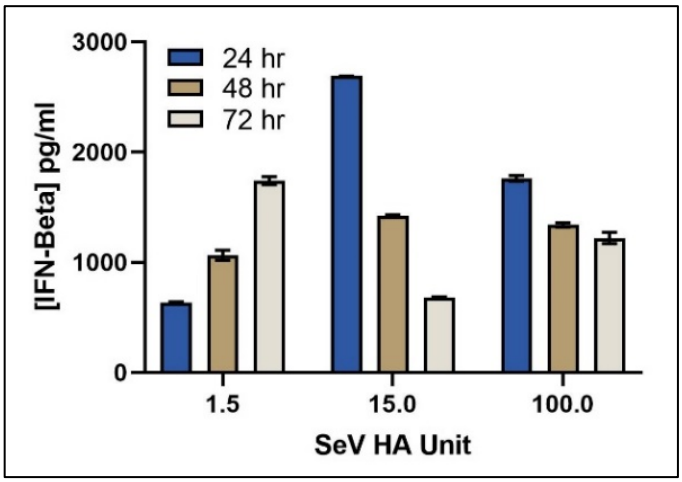


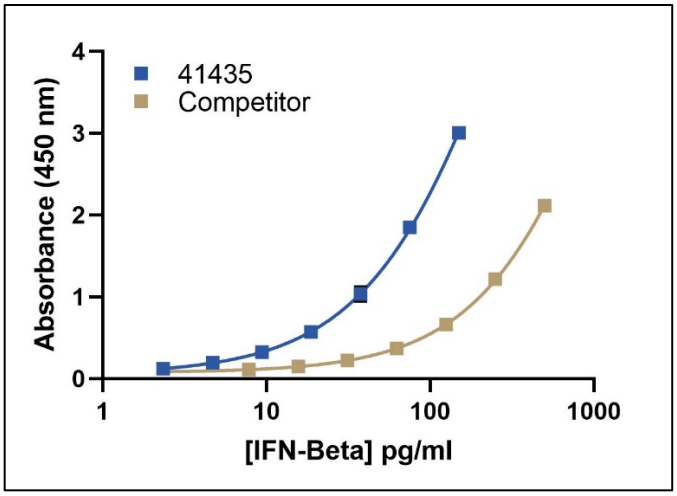
Figure 5. Endogenous Levels of IFN-Beta Quantified in Sendai Virus-Induced HEK293 Cells HEK293 cells were infected with 1.5, 15 and 100 HA units of Sendai Virus (SeV). Supernatants were collected at four time points post-infection (6, 24, 48 and 72 hours). The 6 hr time point is not shown. Due to high expected IFN-Beta levels, samples were diluted 1:100 in TCM. Each uninfected control was assayed and added neat on the plate. All uninfected samples (negative controls) yielded OD values similar to the plate blank of 0.05 indicating no quantifiable IFN-Beta. The standard curves and samples were run in duplicate. Sample concentrations were interpolated from the assay calibrator. Error bars indicate standard deviation.



COMPETITOR KIT PERFORMANCE CHARACTERIZATION

Summary The 41435 ELISA was internally tested against Competitor A. 41435 exhibits higher sensitivity and parallelism (down to the 32-fold dilution) than the competitor kit, and runs over a shorter total assay time.

Figure 7. Representative Standard Curves Competitor A's immunoassay standard curve showcases less sensitivity than PBL's 41435.



VeriKine-HS Human Interferon Beta TCM ELISA Kit (Cat. No. 41435-1)

Technical Data Sheet

Assay Range: 2.34 - 150 pg/ml
Compatibility: Tissue Culture Media (TCM)
Assay Length: 3 hr

Figure 8. sIFNAR2 Receptor Interference in Competitor A's ELISA To test whether soluble Interferon Alpha/Beta Receptor 2 (sIFNAR2) interferes with the detection of IFN-Beta in Competitor A's ELISA, various concentrations of IFN-Beta standard were pre-incubated with and without 10 ug/ml of active sIFNAR2 in Calibrator Diluent (CD). Standards were assayed in duplicate. 0.1% BSA was added as a carrier protein to the Calibrator Diluent with and without sIFNAR2. Data was generated from three runs.

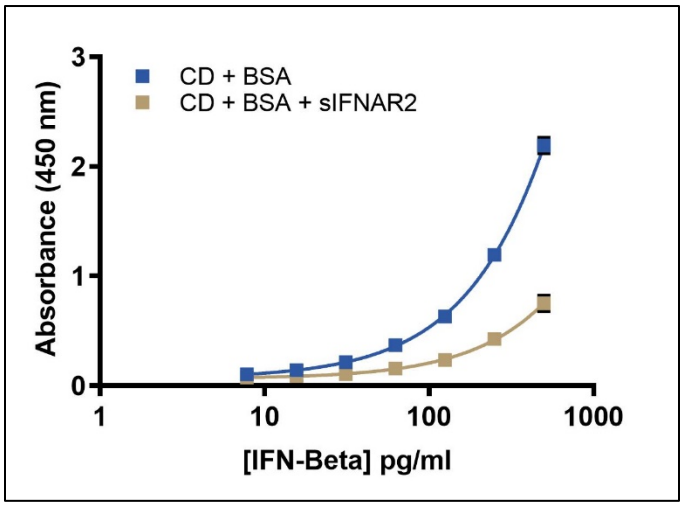


Figure 9. sIFNAR2 Receptor Interference in Competitor A's ELISA The recovery of Human IFN-Beta Standard is shown at different concentrations in Calibrator Diluent (CD) both with and without sIFNAR2. The CD+BSA curve was used as reference for calculating the percent recovery of IFN-Beta in CD+BSA+sIFNAR2 vs the expected value. Data was generated from three runs. Error bars indicate the standard deviation. Incubation with 10 ug/ml of active sIFNAR2 elicited 80% suppression of the detection of IFN-Beta in Competitor A's ELISA.

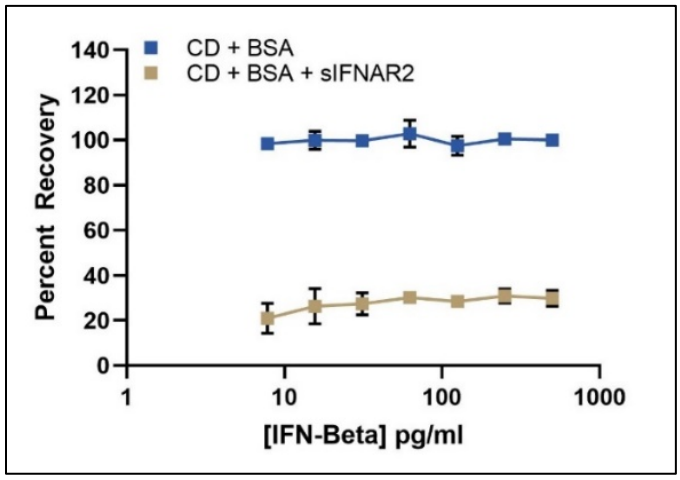


Figure 10. sIFNAR2 Receptor Interference in PBL's 41435 To test whether sIFNAR2 interferes with the detection of IFN-Beta in the 41435 ELISA, various concentrations of IFN-Beta standard were pre-incubated with and without 10 ug/ml of active sIFNAR2. Standards were assayed in duplicate, without 0.1% BSA, in Standard Diluent (SD). 0.1% BSA was added as a carrier protein to the Standard Diluent with and without sIFNAR2. Data was generated from a single run and exhibits a lack of sIFNAR2 receptor interference.

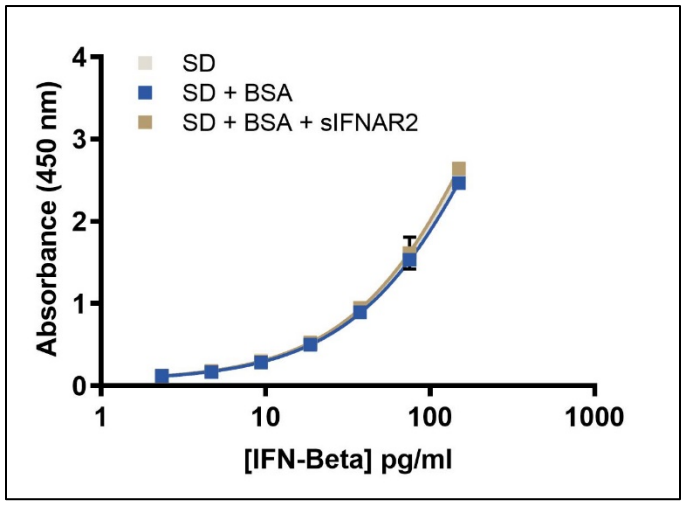


Figure 11. Quantitation of Total IFN-Beta in the presence of sIFNAR2 The recovery of Human IFN-Beta Standard is shown at different concentrations in Standard Diluent (SD) both with and without sIFNAR2, and without 0.1% BSA. The SD curve was used as reference for calculating the percent recovery of IFN-Beta in SD+BSA+sIFNAR2 and SD+BSA vs the expected value. Recoveries of the SD+BSA+ sIFNAR2 curve based on the backfit of SD+BSA curve were similar to recoveries in SD without BSA. Detection of IFN-Beta was neither enhanced nor inhibited by sIFNAR2 at 10 ug/ml. The 41435 ELISA detects free IFN-Beta as well as IFN-Beta bound to soluble IFNAR2 receptors and therefore quantifies a measure of total IFN-Beta. Data was generated from a single run. Error bars indicate the standard deviation.