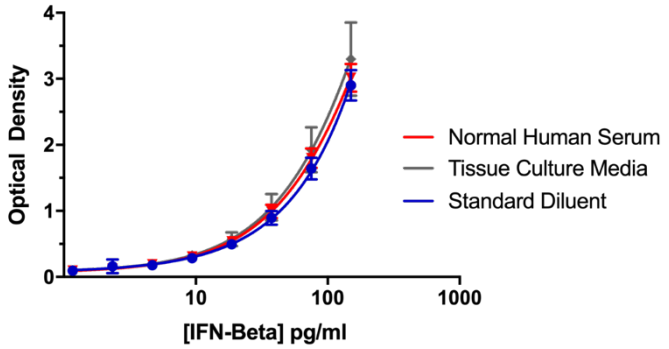
VeriKine-HS™ Human Interferon Beta Serum ELISA Kit
Technical Data Sheet

Assay Range: 1.20 - 150 pg/ml
Compatibility: Serum, Tissue Culture Media (TCM)
Assay Length: 3 hr 30 min

INTRODUCTION

Interferon beta (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. IFN-Beta is biologically unique when compared to other interferons and studies have shown that IFN-Beta has overlapping and distinct gene expression patterns as compared to IFN-Alpha. It appears that IFN-Beta binds to the Type I IFN receptor with higher affinity than the other Type I IFNs and it may also regulate receptor internalization in a different manner. Additionally, IFN-Beta has long been known to inhibit viral replication as part of the body's innate antiviral response and is used as a therapeutic for treatment of multiple sclerosis and some tumors.

Figure 1. Typical Standard Curves in Various Matrices Human IFN-Beta ELISA standard curves were prepared in normal human serum (NHS), tissue culture media (TCM) and standard diluent (SD). The graph below shows the mean of 8 runs in standard diluent and NHS over multiple plates, as well as the mean of 4 runs in TCM. Error bars indicate the standard deviation of the mean.



Specifications This kit quantitates human IFN-Beta in sera, plasma and tissue culture media by sandwich enzyme linked immunosorbent assay (ELISA). Interferon binds to plates coated with antibody and detection is accomplished using a biotinylated secondary antibody followed by streptavidin conjugated to horseradish peroxidase (HRP). Tetramethylbenzidine (TMB) is the substrate. The standard provided is recombinant human IFN-Beta expressed in mammalian cells.

Specificity Human IFN-Beta. No cross-reactivity detected with human IFN-Alpha, human IFN-Gamma, human IFN-Omega or human IL-6. No cross-reactivity with mouse IFN-Alpha, mouse IFN-Beta or rat IFN-Beta.

PRECISION

Figure 2. Intra-Assay and Inter-Assay CV To test precision within and between assays, a series of 8-point standard curves prepared in either standard diluent or a human serum pool was assayed in duplicate. A total of 8 assays were applied for comparison.

	Intra- and Inter-Assay Precision	
Matrix Type	**SD**	**Serum Pool**
n	8	8
Intra-Assay CV (%)	3.1	2.5
Inter-Assay CV (%)	1.8	1.1

SPIKE RECOVERY

Figure 3. Spike Recovery Human IFN-Beta was spiked into **Healthy Donor Serum** at three known concentrations in eight independent assays.

	Spike Recovery		
Spike Level	**1**	**2**	**3**
Target Conc. (pg/ml)	100	20	4
Avg Recovery (%)	100.2	94.8	88.0
Range (%)	94.8 - 106.3	82.3 - 104.4	70.3 - 106.8

Figure 4. Spike Recovery Human IFN-Beta was spiked into **Healthy Donor EDTA Plasma** at three known concentrations within one independent assay.

	Spike Recovery		
Spike Level	**1**	**2**	**3**
Target Conc. (pg/ml)	100	25	5
Avg Recovery (%)	97.7	92.8	101.2
Range (%)	93.5 - 103.9	90.6 - 95.9	93.5 - 120.4

Figure 5. Spike Recovery Human IFN-Beta was spiked into **Healthy Donor Heparin Plasma** at three known concentrations within two independent assays.

	Spike Recovery		
Spike Level	**1**	**2**	**3**
Target Conc. (pg/ml)	100	25	5
Avg Recovery (%)	86.9	88.4	97.6
Range (%)	83.4 - 94.2	83.6 - 94.7	84.2 - 115.6

Figure 6. Spike Recovery Human IFN-Beta was spiked into **Healthy Donor Citrate Plasma** at three known concentrations within one independent assay.

	Spike Recovery		
Spike Level	**1**	**2**	**3**
n	100	25	5
Avg Recovery (%)	87.2	87.6	92.7
Range	78.2 - 97.0	75.5 - 100.0	82.4 - 106.9

PERFORMANCE CHARACTERIZATION

Figure 7. Lot-to-Lot Consistency Standard curves from 8 different lots of 41415 Human IFN-Beta Standard were evaluated and compared to a recent lot of 41415 (L-6966). Expired kit lots (denoted by italics and an asterisk (*)) were tested as part of an ongoing stability study. L-6790 and L-6672 were tested within a year of expiration, L-6428 and L-6530 at 1 year post-expiration, and L-6277 at 2 years post-expiration. IFN-Beta was detectable with an accuracy of +/- 20% of the mean calculated concentration. Error bars indicate the standard deviation between two runs. All concentrations were run twice with the exception of 150 and 1.17 pg/ml.

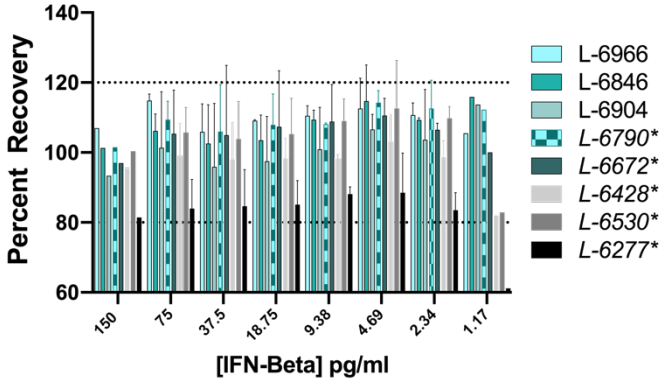


Figure 8. Linearity of Dilution in Normal Human Serum (NHS) 5 independent spikes of 80 pg/ml were prepared using one low background human serum lot. Five two-fold serial dilutions of the spike were performed in standard diluent. Spikes were tested over 3 different days. The concentrations of the variably diluted spikes were calculated from the standard curve in standard diluent. IFN-Beta was detectable with an accuracy of +/- 20% of the expected value with the exception of a single dilution from one run.

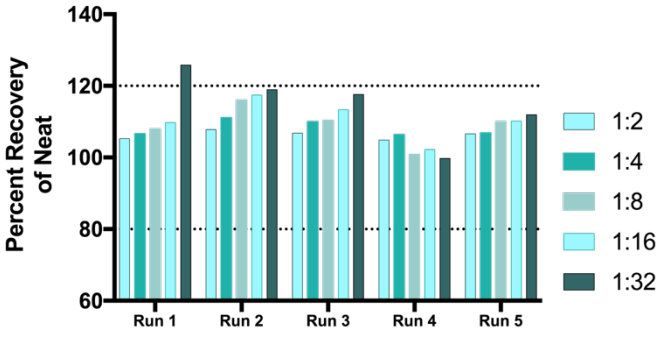


Figure 9. Linearity of Dilution in Multiple Sclerosis (MS) Samples Assay linearity was tested in 4 different sera from individual MS patients undergoing IFN-Beta therapy. All samples had been previously tested for IFN-Beta. All samples were run in a single experiment with the exception of Sample 6276 (±) which was run twice. Six or seven two-fold serial dilutions were made of each serum sample in standard diluent. The concentrations of IFN-Beta were calculated from the standard curve in standard diluent. IFN-Beta was detectable with an accuracy of +/- 20% of the neat calculated concentration.

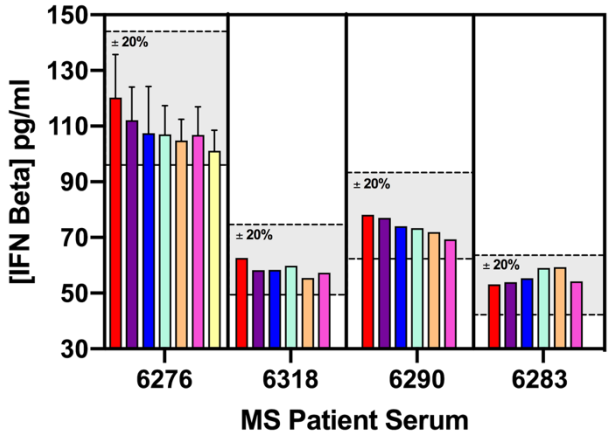
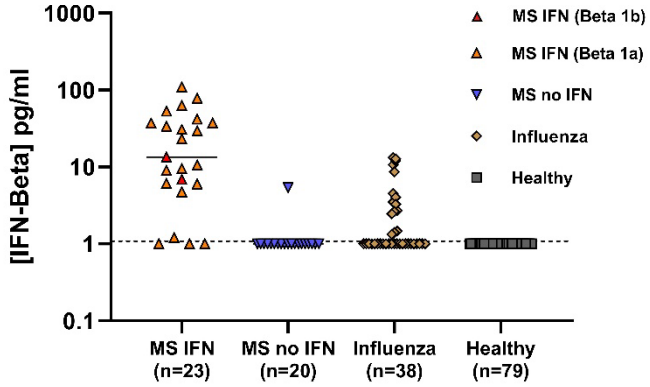


Figure 10. Levels of IFN-Beta Quantified in Healthy Donor Sera (HDS), Influenza Patient Sera, and Multiple Sclerosis (MS) Patient Sera 43 MS serum samples, 38 Influenza serum samples, and 79 NHS samples were tested for IFN-Beta. 23 MS patients were on IFN-Beta treatment and 20 were on other therapies. Of the 23 MS patients on IFN-Beta treatment, 2 were on Betaseron (Beta 1b), while the remaining 21 were on Rebif and Avonex (Beta 1a). IFN-Beta was quantifiable in 87% of MS patients on IFN-Beta therapy, 5% of patients on non-IFN therapies, and 40% of Influenza patients. All healthy donors presented IFN-Beta values under the assay LLOQ. All samples found to be below the LLOQ are plotted on the graph below at the level of 1.0 pg/ml.



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