VeriKine-HS™ Human Interferon Beta Serum ELISA Kit
Certificate of Analysis & Protocol B
(For Increased Serum or Plasma Sensitivity, Please Follow Protocol A)

Assay Range: 2.3 - 150 pg/ml Compatibility: Serum, Plasma, Tissue Culture Media (TCM)
Assay Length: 3 hr

Catalog No: 41415-1
Lot No:
Expiration:
Store all components at 2-8ºC

Kit Components	Part No.	Lot No.	Quantity
Plate(s)	SMP138		1
Plate Sealers	N/A	N/A	4
Wash Solution Concentrate	SMP057-60		2 x 50 ml
Human IFN-Beta Standard, 100,000 pg/ml	SMP146-1		1 vial
Standard Diluent	SMP163-30		25 ml
Sample Buffer	SMP147-15		15 ml
Antibody Concentrate	SMP148-1		1 vial
HRP Conjugate Concentrate	SMP056-320		1 vial
Assay Diluent	ASD-30		25 ml
TMB Substrate Solution	KET-15		15 ml
Stop Solution	SCY-15		15 ml

Product Performance Specifications

	Standard Diluent	Human Serum	TCM (10% FBS)
Intra-Assay CV	≤ 8%	≤ 6%	≤ 8%
Inter-Assay CV	≤ 10%	≤ 10%	≤ 10%

Authorization

Released by: _____

Date:

NOTE: Methods associated with collection, storage and testing of experimental samples have all been reported to affect ELISA results. Although extensive testing has been carried out to minimize sample matrix effects, the user should determine whether the test sample matrix adversely affects recovered IFN-β values.

PIPETTING TIPS: Due to the inherent nature of human IFN-β protein to adhere to plastic surfaces, proper pipetting technique is required to accurately prepare a standard curve and quantitate samples.

Aspirating: To avoid protein sticking to outside walls of the pipette tip, ensure it is not immersed in the standard vial when aspirating.

Dispensing and Diluting: Proper mixing technique entails pipetting up and down gently 10 times for predilution and S7 dilution; 5 times for subsequent serial dilutions. Thorough, but gentle, pipetting is required to recover all material attached to the inside of the tip. Avoid excessive force or foaming to prevent denaturing.

CAUTION: Certain kit components are considered hazardous and should be handled with appropriate safety precautions and discarded properly. For specific information, consult the safety data sheet (SDS).

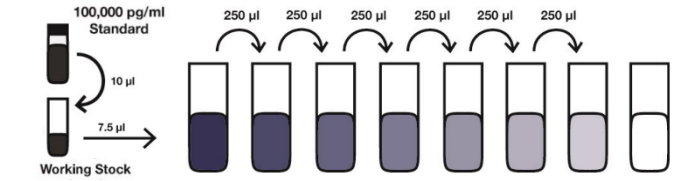
PREPARATION OF REAGENTS

Wash Solution: Wash Solution Concentrate may contain crystals; place in a warm water bath and gently mix until completely dissolved. Prepare a 1:10 working wash solution (e.g. Add 50 ml Wash Solution Concentrate to 450 ml distilled or deionized water). Mix thoroughly before use. Store at RT (22-25ºC).

Human IFN-Beta Standard Curve Preparation:

a. Label seven polypropylene tubes (S1 – S7).
b. Add indicated volume of Standard Diluent or Sample Matrix to each tube as indicated in Figure 1.
c. Prepare *working stock* by pipetting 10 µl Standard into 90 µl Standard Diluent or Sample Matrix. Using 100 or 200 µl pipette, set the volume to 80 µl and mix thoroughly.
d. Using polypropylene tips, add 7.5 µl of prediluted standard to S7 and mix thoroughly. Remove indicated amount from S7, add to S6, and mix thoroughly. Repeat to complete series to S1.

Figure 1: 7-Point Standard Curve Prepared in Standard Diluent



Label	S7	S6	S5	S4	S3	S2	S1	Blank
Dilution Matrix (µl)	492.5	250	250	250	250	250	250	250
IFN-Beta (pg/ml)	150	75	37.5	18.8	9.4	4.7	2.3	0

Sample Preparation: Thaw frozen sample tubes to Room Temperature (RT) (22-25ºC) in either tap water or between the fingertips. If samples require dilution, prepare using Standard Diluent or Sample Matrix. Keep at RT until use. Measurements in duplicate are recommended.

Antibody Solution: Prior to use, dilute Antibody Concentrate in the volume of recommended Assay Diluent as shown below. Keep at RT until use.

Micro-plate Strips Used	2	4	6	8	10	12
Antibody Concentrate (µl)						
Assay Diluent (ml)	2.0	3.0	4.0	5.0	6.0	7.0

HRP Solution: 15 minutes prior to use in step 2, dilute HRP Conjugate Concentrate in the volume of Assay Diluent as shown below. Keep at RT until use.

Micro-plate Strips Used	2	4	6	8	10	12
HRP Conjugate Concentrate (µl)						
Assay Diluent (ml)	3.0	5.0	6.0	8.0	10.0	12.0

ASSAY PROCEDURE

Bring to RT (22-25ºC)	Keep at 2-8ºC
Plate/Sealers	Human IFN-Beta Standard
Sample Buffer	Antibody Concentrate
Standard Diluent	HRP Conjugate Concentrate
Assay Diluent	
TMB Substrate Solution	
Stop Solution	
Matrices/Samples	

- **Incubations:** All incubations should be conducted in a closed chamber at RT, keeping the plate away from drafts.
- **Plate Washing**: All wells should be filled with a minimum of 300 µl of Wash Solution. Remove plate contents by inverting and blotting the plate on lint-free absorbent paper; tap the plate dry.

1. Determine the number of microplate strips required. We recommend running both the standard and samples at least in duplicate. Remove extra microtiter strips from the frame, seal in the foil bag provided and store at 2-8ºC. Unused strips can be used in later assays.

Total well volume = 150 µl (Step A + Step B + Step C)
Step A: Add **50 µl** of **Sample Buffer** to every well.
Step B: Add **50 µl** of diluted **Antibody Solution** to each well.
Step C: Add **50 µl** of diluted **Standard**, **Test Samples** or **Blanks** (Standard Diluent or appropriate dilution matrix) to each designated well.

Cover with Plate Sealer and shake at 450 rpm at RT for 2 hours.

After 2 hours, empty plate contents and wash wells three times.

2. Add **100 µl** of diluted **HRP Solution** to each well. Cover with Plate Sealer and shake plate at 450 rpm at RT for 30 minutes.

After 30 minutes, empty plate contents and wash wells four times.

3. Add **100 µl** of **TMB Substrate Solution** to each well. Incubate **in the dark** at RT for 30 min. Do not use a Plate Sealer and DO NOT SHAKE during the incubation.

4. After 30 minutes, DO NOT EMPTY THE WELLS AND DO NOT WASH. Add **100 µl** of **Stop Solution** to each well.

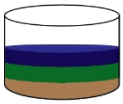
5. Using a microplate reader, determine the absorbance at 450 nm within 5 minutes after the addition of Stop Solution.

Visit PBL's website (https://pblassaysci.com/documentation) for additional information including technical data sheet

HUMAN IFN-BETA ELISA (41415)
ASSAY PROCEDURE – QUICK REFERENCE

Total Time: 3 hr

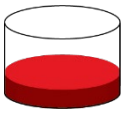
Note: All incubations are at Room Temperature (RT) (22-25ºC)*



1. Add **50 µl** Sample Buffer
2. Add **50 µl** Diluted Antibody Solution
3. Add **50 µl** Standard, Sample or Blank

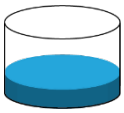
Incubate **2 hr** *(shake at 450 rpm) at RT**

Aspirate and Wash **3x**

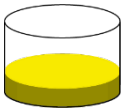


Add **100 µl** diluted HRP Solution
Incubate **30 min** *(shake at 450 rpm) at RT**

Aspirate and Wash **4x**



Add **100 µl** TMB Substrate
Incubate **30 min** *in the dark*
Do not seal, shake or wash.

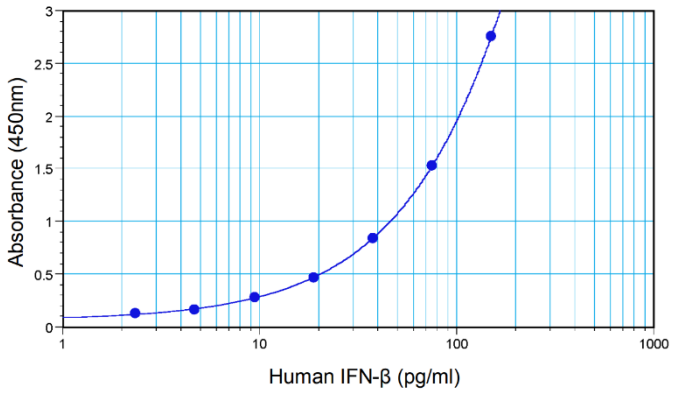


Add **100 µl** Stop Solution
Read plate within 5 min (450 nm)

CALCULATION OF RESULTS

By plotting the optical densities (OD) using a 4-parameter fit for the standard curve, the interferon titer in the samples can be determined. Blank ODs may be subtracted from the standards and sample ODs to eliminate background. Use the conversion factor of 3 pg/unit to approximate titers in units/ml.

Figure 2: Typical Standard Curve in Standard Diluent



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