VeriKine-HS™ Human Interferon Beta TCM ELISA Kit
Certificate of Analysis & Protocol

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

Catalog No: 41435-1
Lot No: 7528
Expiration: June 30, 2023
Store all components at 2-8ºC

Kit Components	Part No.	Lot No.	Quantity
Plate(s)	SMP138	K6932	1
Plate Sealers	N/A	N/A	4
Wash Solution Concentrate	SMP057-60	K6742	2 x 50 ml
Human IFN-Beta Standard, 100,000 pg/ml	SMP146-1	K6935	1 vial
Standard Diluent	SMP163-30	K6938	25 ml
Sample Buffer	SMP147-15	K6909	15 ml
Antibody Concentrate	SMP148-1	K6936	1 vial
HRP Conjugate Concentrate	SMP056-320	K6937	1 vial
Assay Diluent	ASD-30	314897	25 ml
TMB Substrate Solution	KET-15	210201D01	15 ml
Stop Solution	SCY-15	62731	15 ml

Authorization

Released by: _____

Date: June 21, 2022

Note: The 41435-1 ELISA is not intended for use with human serum or plasma samples because of a significantly elevated risk of false positive results.

Visit the product page on PBL's website (https://pblassaysci.com) to view the technical supplement and additional product information.

CAUTION: Components should be handled with appropriate safety precautions and discarded properly. For further information, consult the safety data sheet (SDS).

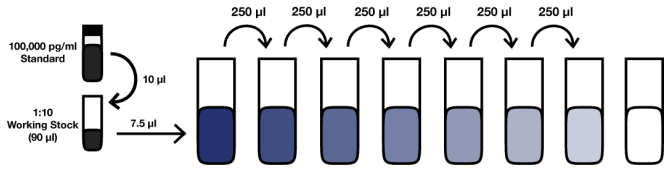
PREPARATION OF REAGENTS

Wash Buffer: 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. (**Note:** Prepare fresh Wash Buffer for each assay run.)

Human IFN-Beta Standard Curve Preparation:

a. Label seven polypropylene tubes (S1 – S7).
b. Add volume of Standard Diluent or Sample Matrix to each tube as indicated in Figure 1.
c. Using polypropylene tips, add 10 µl of the Human IFN-Beta Standard to 90 µl of Standard Diluent or Sample Matrix.
d. Add 7.5 µl of prediluted standard to S7 and mix thoroughly.
e. Transfer 250 µl of S7 to S6 and mix thoroughly. Repeat to complete series to S1. Set aside until use in step 1.

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



Label	S7	S6	S5	S4	S3	S2	S1	Blank
Standard Diluent (µl)	492.5	250	250	250	250	250	250	250
IFN-Beta (pg/ml)	150.00	75.00	37.50	18.75	9.38	4.69	2.34	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 in step 1, dilute Antibody Concentrate in the volume of Assay Diluent as shown below. Keep at RT (22-25ºC).

Micro-plate Strips Used	**2**	**4**	**6**	**8**	**10**	**12**
Antibody Concentrate (µl)	13	19	26	32	38	45
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 (22-25ºC).

Micro-plate Strips Used	**2**	**4**	**6**	**8**	**10**	**12**
HRP Conjugate Concentrate (µl)	24	40	48	64	80	96
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
Matrices/Samples	
Assay Diluent	
TMB Substrate Solution	
Stop Solution	

- **Incubations:** Use plate sealers to cover the plate when directed. All incubations should be conducted in a closed chamber at 22-25ºC or 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 A: Add **50 µl** of **Sample Buffer** and **50 µl** of diluted **Antibody Solution** to every well.
Step B: Add **50 µl** of **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 at 450 rpm at RT for 30 minutes.

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

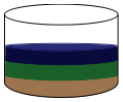
3. Add **100 µl** of **TMB Substrate Solution** to each well. Incubate **in the dark** at RT for 30 minutes. Do not use a Plate Sealer during the incubation. DO NOT SHAKE.

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.

HUMAN IFN-BETA ELISA (41435)
ASSAY PROCEDURE – QUICK REFERENCE

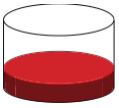
Total Time: 3 hr
Note: All incubations are at Room Temperature (RT) (22-25ºC)*



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

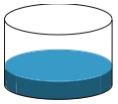
Incubate **2 hr** *(shake at 450 rpm) at RT**

Aspirate and Wash **3x**

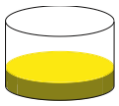


Add **100 µl** diluted HRP Solution
Incubate **30 min** *(shake at 450 rpm) at RT**

Aspirate and Wash **4x**



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

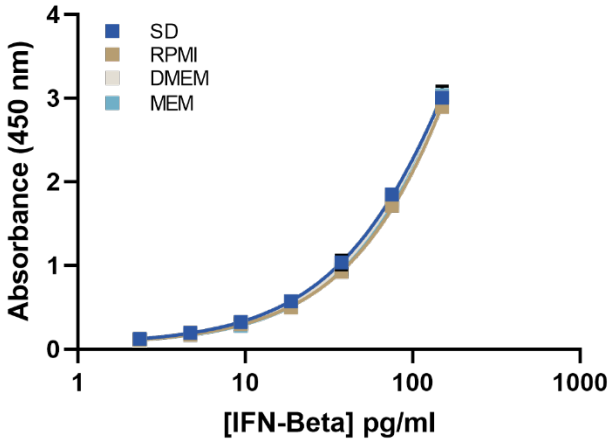


Add **100 µl** Stop Solution
Read plate within 5 min (450 nm)

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 conversion factor of 3 pg/unit to approximate titers in units/ml. A 4-parameter logistic plot with $1/y^2$ weighted analysis is recommended for obtaining optimal fit of standard curve OD values.

Figure 2: Typical Standard Curve



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131 Ethel Road West, Suite 6 | Piscataway, NJ 08854 USA | T: +1.732.777.9123 | F: +1.732.777.9141 | E: info@pblassaysci.com | W: pblassaysci.com