VeriKine-HS™ Mouse Interferon Beta Serum ELISA Kit
Certificate of Analysis & Protocol

Assay Range: 0.94 – 60 pg/ml
Compatibility: Serum, Plasma, Tissue Culture Media (TCM)
Assay Length: 1 hr 50 min

Catalog No: 42410-1
Lot No: 7530
Expiration: June 30, 2023
Store all components at 2-8ºC

Kit Components	Part No.	Lot No.	Quantity
Plate(s)	SMP199	K6942	1
Plate Sealers	N/A	N/A	4
Wash Solution Concentrate	SMP057-60	K6742	2 x 50 ml
Mouse IFN Beta Serum Standard, 10,000 pg/ml	SMP195-1	K6945	1 vial
Sample Diluent	SMP196-30	K6946	25 ml
Antibody Concentrate	SMP197-1	K6947	1 vial
HRP Conjugate Concentrate	SMP056-150	K6950	1 vial
Antibody Diluent	SMP198-15	K6949	15 ml
HRP Diluent	ASDHRP-15	648946	15 ml
Serum Buffer	SMP213-15	K6948	12 ml
TMB Substrate Solution	KET-15	210201D01	15 ml
Stop Solution	SCY-15	59172	15 ml

Authorization

Released by: _____

Date: July 12, 2022

Visit the product page on PBL's website (https://pblassaysci.com) to view the full protocol, including performance characterization and kit specifications.

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

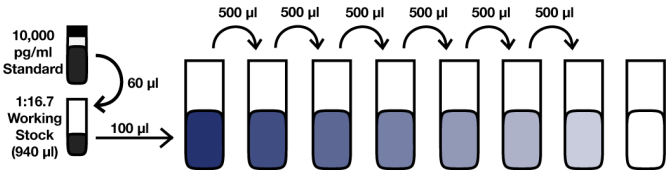
PREPARATION OF REAGENTS

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 of distilled or deionized water). Mix thoroughly before use.

Mouse IFN Beta Standard Curve Preparation:

a. Prepare a 1:16.7 *working stock* of Mouse IFN-β Standard by pipetting 60 µl of Standard into 940 µl of Sample Diluent. Mix thoroughly by gently pipetting up and down 10 times.
b. Label seven polypropylene tubes (S1 – S7).
c. Add indicated volume of Sample Diluent to each tube as indicated in Figure 1.
d. Add 100 µl of *working stock* to S7 and mix thoroughly to recover all material adhered to the inside of the pipette tip.
e. Using a pipette set at 500 µl, mix S7 thoroughly. Remove indicated volume from S7 and add to S6. Mix thoroughly. Repeat to complete series to S1.
f. Set aside on ice (2-8ºC) until step 1.

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



Label	S7	S6	S5	S4	S3	S2	S1	Blank
Sample Diluent (µl)	900	500	500	500	500	500	500	500
IFN-β (pg/ml)	60	30	15	7.5	3.75	1.87	0.94	0

Sample Preparation: Thaw frozen samples to Room Temperature (RT) (22-25ºC) in either tap water or between the fingertips. If samples require dilution, prepare using Sample Diluent. Keep on ice (2-8ºC) until step 1. Measurements in duplicate are recommended.

Antibody Solution: 15 minutes prior to use in step 3, dilute Antibody Concentrate in the volume of Antibody Diluent shown below. Keep on ice (2-8ºC) until use.

Micro-plate Strips Used	2	4	6	8	10	12
Antibody Concentrate (µl)	33	50	67	83	100	117
Antibody Diluent (ml)	2.0	3.0	4.0	5.0	6.0	7.0

HRP Solution: 15 minutes prior to use in step 4, dilute HRP Concentrate in the volume of HRP Diluent shown below. Keep on ice (2-8ºC) until use.

Micro-plate Strips Used	2	4	6	8	10	12
HRP Conjugate Concentrate (µl)	29	43	57	71	86	100
HRP Diluent (ml)	2.0	3.0	4.0	5.0	6.0	7.0

ASSAY PROCEDURE

Bring to RT (22-25ºC)	Keep at 2-8ºC
Plate	Mouse IFN Beta Standard
Plate Sealers	Sample Diluent
Wash Solution Concentrate	Antibody Concentrate
Serum Buffer	HRP Conjugate Concentrate
TMB Substrate Solution	Antibody Diluent
Stop Solution	HRP Diluent

- **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 250 µl of Wash Buffer. 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.

2. Total well volume = 100 µl (Step A + Step B)
Step A: Add **50 µl** of **Serum Buffer** (for serum or plasma samples) OR **Sample Diluent** (for tissue culture samples) to every well.
Step B: Add **50 µl** of diluted **Standard**, **Test Samples** or **Blanks** (Sample Diluent or appropriate dilution matrix) to each designated well.

Cover with Plate Sealer and shake at 650 rpm at RT for 1 hour.

After 1 hour, empty plate contents and wash wells four times.

3. Add **50 µl** of diluted **Antibody Solution** to each well. Cover with Plate Sealer and shake plate at 650 rpm at RT for 30 minutes.

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

4. Add **50 µl** of diluted **HRP Solution** to each well. Cover with Plate Sealer and shake plate at 650 rpm at RT for 10 minutes.

After 10 minutes, empty the plate contents and wash wells four times.

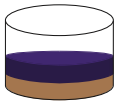
5. Add **100 µl** of **TMB Substrate Solution** to each well. Incubate **in the dark** at RT for 10 minutes. Do not use a Plate Sealer and DO NOT SHAKE during the incubation.

6. After 10 minutes, DO NOT EMPTY THE WELLS AND DO NOT WASH. Add **100 µl** of **Stop Solution** to each well.

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

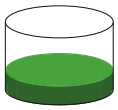
MOUSE IFN BETA SERUM ELISA (42410)
ASSAY PROCEDURE – QUICK REFERENCE

Total Time: 1 hr 50 min
*RT: Room Temperature (22-25ºC)
Note: All incubations are at RT



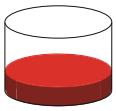
1. Add **50 µl Serum Buffer** (for serum or plasma samples) OR **Sample Diluent** (for tissue culture samples)
2. Add **50 µl** Standard, Sample or Blank
Incubate **1 hr** *(shake at 650 rpm) at RT**

Aspirate and Wash **4x**



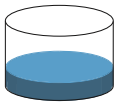
Add **50 µl** diluted **Antibody Solution**
Incubate **30 min** *(shake at 650 rpm) at RT**

Aspirate and Wash **4x**

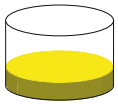


Add **50 µl** diluted **HRP Solution**
Incubate **10 min** *(shake at 650 rpm) at RT**

Aspirate and Wash **4x**



Add **100 µl TMB Substrate Solution**
Incubate **10 min** *in the dark at RT**
Do not seal, shake or wash.

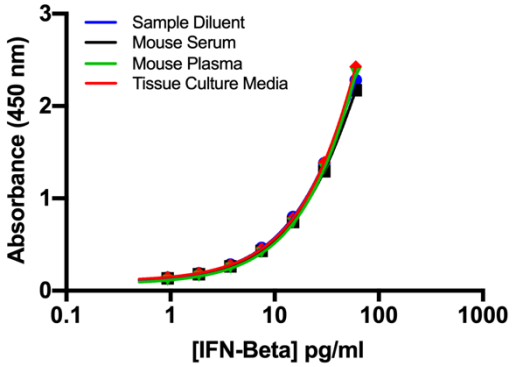


Add **100 µl Stop Solution**
Read plate within 5 min (450 nm)

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.

Figure 2: Typical Standard Curves in Various Matrices



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