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

Assay Range: 15.6 - 1000 pg/ml
Compatibility: Serum, Tissue Culture Media
Assay Length: 3 hr 15 min

Catalog No: 41110-1
Lot No:
Expiration:
Store all components at 2-8ºC

Kit Components	Part No.	Lot No.	Quantity
Plate(s)	SMP028		1
Plate Sealers	N/A	N/A	4
Wash Solution Concentrate	SMP022-60		50 ml
Mouse IFN-Beta Standard, 500,000 pg/ml	SMP210-1		1 vial
Dilution Buffer	SMP021-30		30 ml
Antibody Concentrate	SMP211-1		1 vial
HRP Conjugate Concentrate	SMP179-240		1 vial
Concentrate Diluent	SMP024-30		30 ml
TMB Substrate Solution	KET-15		15 ml
Stop Solution	SCY-15		15 ml

Product Performance Specifications

Intra-Assay CV	< 8%
Inter-Assay CV	< 8%

Authorization

Released by: _____

Date:

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

CAUTION: Dilution Buffer, Wash Solution Concentrate and Concentrate Diluent contain 0.1% Kathon CG/ICP as a preservative and should be handled with appropriate safety precautions and discarded properly. For further 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 gentl y 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.

Mouse IFN-Beta Standard Curve Preparation:

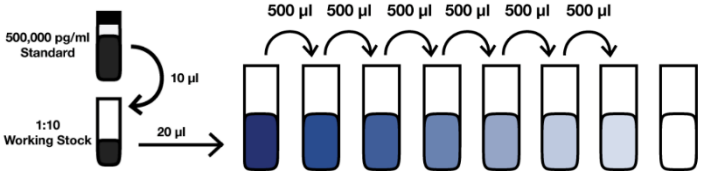
a. Label seven polypropylene tubes (S1 – S7).

b. Prepare *working stock* by pipetting 10 µl Standard into 90 µl Dilution Buffer or Sample Matrix. Mix thoroughly by gently pipetting up and down 10 times.

c. Add indicated volume Dilution Buffer to each tube as indicated in Figure 1.

d. Using polypropylene tips, add indicated amount of *working stock* to S7. Use a pipette set at 500 µl and mix thoroughly. Remove indicated amount from S7 and add to S6. Repeat to complete series to S1. *Change tips between each dilution.*

Figure 1: 7-Point Standard Curve Prepared in Dilution Buffer



Label	S7	S6	S5	S4	S3	S2	S1	Blank
Dilution Buffer (µl)	980	500	500	500	500	500	500	500
IFN-Beta (pg/ml)	1000	500	250	125	62.5	31.3	15.6	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 Dilution Buffer or Sample Matrix. Keep at RT until use. Measurements in duplicate are recommended.

Antibody Solution: 15 minutes prior to use, dilute Antibody Concentrate in volume of Concentrate Diluent shown below. Keep at RT (22-25ºC).

Micro-plate Strips Used	**2**	**4**	**6**	**8**	**10**	**12**
Antibody Concentrate (µl)						
Concentrate Diluent (ml)	2.0	4.0	6.0	8.0	10.0	12.0

HRP Solution: 15 minutes prior to use, dilute HRP Conjugate Concentrate in volume of Concentrate Diluent shown below. Keep at RT (22-25ºC).

Micro-plate Strips Used	**2**	**4**	**6**	**8**	**10**	**12**
HRP Conjugate Concentrate (µl)						
Concentrate Diluent (ml)	2.0	4.0	6.0	8.0	10.0	12.0

ASSAY PROCEDURE

Bring to RT (22-25ºC)	Keep at 2-8ºC
Plate/Sealers	Mouse IFN-Beta Standard
Dilution Buffer	Antibody Concentrate
Matrices/Samples	HRP Conjugate Concentrate
Concentrate Diluent	
TMB Substrate	
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 250 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.

Add **100 µl** of **Standard**, **Sample** or **Blank** (Dilution Buffer or appropriate dilution matrix) to each designated well.

Cover with Plate Sealer and incubate at RT for 1 hour.

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

2. Add **100 µl** of diluted **Antibody Solution** to each well.

Cover with Plate Sealer and incubate at RT for 1 hour.

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

3. Add **100 µl** of **HRP Solution** to each well.

Cover with Plate Sealer and incubate at RT for 1 hour.

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

4. Add **100 µl** of **TMB Substrate Solution** to each well. Incubate **in the dark** at RT for 15 minutes. Do not use a Plate Sealer during the incubation.

5. After 15 minutes, DO NOT EMPTY THE WELLS AND DO NOT WASH. Add **100 µl** of **Stop Solution** to each well.

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

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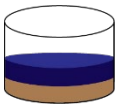
MOUSE IFN-BETA ELISA (42400)
ASSAY PROCEDURE – QUICK REFERENCE

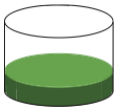
Total Time: 3 hr 15 min

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



Add **100 µl** Standard, Sample, or Blank
Incubate **1 hr** *at RT**

Aspirate and Wash **3x**

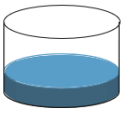


Add **100 µl** diluted Antibody Solution
Incubate **1 hr** *at RT**

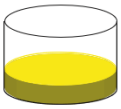
Aspirate and Wash **3x**

Add **100 µl** diluted HRP Solution
Incubate **1 hr** *at RT**

Aspirate and Wash **3x**



Add **100 µl** TMB Substrate
Incubate **15 min** *in the dark at RT**
Do not seal, shake, or wash.

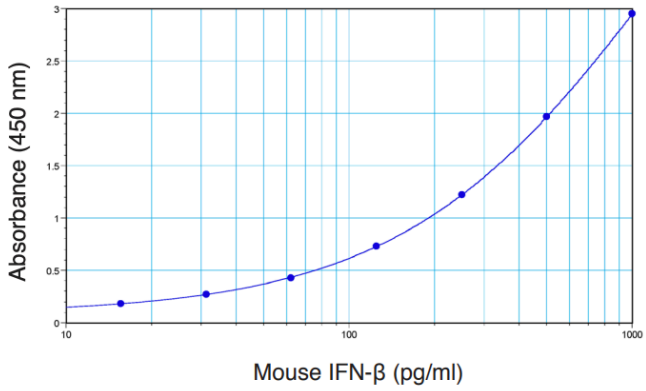


Add **100 µl** Stop Solution
Read plate within 5 min (450 nm)

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 Curve



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