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

Assay Range: 5.47 - 350 pg/ml
Compatibility: Serum, Plasma, Tissue Culture Media (TCM)
Assay Length: 3 hr

Catalog No: 46415-1
Lot No: 7466
Expiration: November 30, 2022
Store all components at 2-8ºC

Kit Components	Part No.	Lot No.	Quantity
Plate(s)	SMP138	K6732	1
Plate Sealers	N/A	N/A	4
Wash Solution Concentrate	SMP057-60	K6742	2 x 50 ml
Cyno IFN Beta Standard, 100,000 pg/ml	SMP261-1	K6740	1 vial
Standard Diluent	SMP163-30	K6739	25 ml
Sample Buffer	SMP147-15	K6736	15 ml
Antibody Concentrate	SMP148-100	K6741	1 vial
HRP Conjugate Concentrate	SMP056-450	K6738	1 vial
Assay Diluent	ASD-30	314897	25 ml
TMB Substrate Solution	KET-15	210201D01	15 ml
Stop Solution	SCY-15	59172	15 ml

Authorization

Released by: _____

Date: November 22, 2021

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).

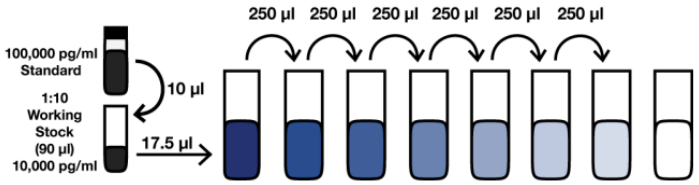
PREPARATION OF REAGENTS

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 distilled or deionized water). Mix thoroughly before use.

Cyno IFN-Beta Standard Curve Preparation:

a. Prepare a 1:10 *working stock* of Cyno IFN-Beta standard by pipetting 10 µl of IFN standard into 90 µl of Standard Diluent or Sample Matrix. Mix thoroughly by gently pipetting up and down 10 times.
b. Label seven polypropylene tubes (S1 – S7).
c. Add indicated volume of Standard Diluent or Sample Matrix to each tube as indicated in Figure 1.
d. Using polypropylene tips, add 17.5 µl of working stock to S7 as indicated and mix thoroughly by pipetting up and down 10 times. 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 Sample Matrix



Label	S7	S6	S5	S4	S3	S2	S1	Blank
Dilution Matrix (µl)	482.5	250	250	250	250	250	250	250
IFN-Beta (pg/ml)	350	175	87.5	43.75	21.88	10.94	5.47	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)	16	24	32	40	48	56
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	All other components
Wash Solution Concentrate	
Standard Diluent	
Sample Buffer	
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 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.

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 diluted **TMB Substrate Solution** to each well. Do not use a Plate Sealer and DO NOT SHAKE during the incubation.

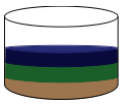
4. After 30 minutes, DO NOT EMPTY THE WELLS AND DO NOT WASH. Add **100 µl** of **Stop Solution** to each well.

5. Using a microplate reader, determine the absorbance at 450 nm within 2 minutes after the addition of Stop Solution.

CYNO IFN-BETA ELISA (46415)
ASSAY PROCEDURE – QUICK REFERENCE

Total Time: 3 hr

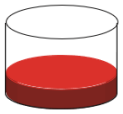
Note: All incubations are at Room Temperature (RT) (22-25ºC)*



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

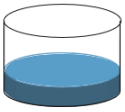
Incubate **2 hr** *(shake at 450 rpm) at RT**

Aspirate and Wash **3x**

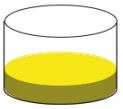


Add **100 µl** diluted HRP Solution
Incubate **30 min** *(shake at 450 rpm) at RT**

Aspirate and Wash **4x**



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

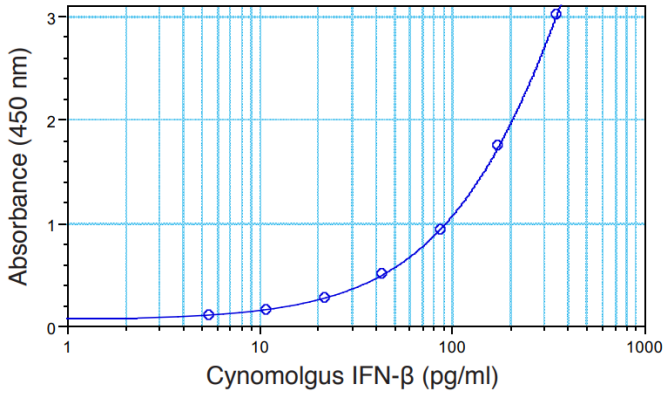


Add **100 µl** Stop Solution
Read plate within 2 min (450 nm)

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 in Standard Diluent



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

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