VeriKine™ Cynomolgus/Rhesus IFN-Alpha Serum ELISA Kit
Certificate of Analysis & Protocol

Assay Range: 25 - 1600 pg/ml
Compatibility: Serum, Plasma, Tissue Culture Media
Assay Length: 3 hr 15 min

Catalog No: 46100-1
Lot No: 7475
Expiration: August 31, 2022
Store all components at 2-8ºC

Kit Components	Part No.	Lot No.	Quantity
Plate(s)	SMP135	K6771	1
Plate Sealers	N/A	N/A	4
Wash Solution Concentrate	SMP022-60	K6719	50 ml
Cyno/Rhesus IFN-Alpha 2 Standard, 10,000 pg/ml	SMP141-1	K6774	1 vial
Standard Diluent	SMP163-30	K6775	25 ml
Sample Buffer	SMP153-8	K6776	8 ml
Antibody Concentrate	SMP154-1	K6777	1 vial
HRP Conjugate Concentrate	SMP056-330	K6778	1 vial
Concentrate Diluent	SMP024-30	K6731	30 ml
TMB Substrate Solution	KET-15	210201D01	15 ml
Stop Solution	SCY-15	59172	15 ml

Product Performance Specifications

	Standard Diluent	Cynomologus Monkey Serum	Tissue Culture Media (10% FBS)
Intra-Assay CV	≤ 8%	≤ 8%	≤ 8%
Inter-Assay CV	≤ 10%	≤ 15%	≤ 10%

Authorization

Released by: _____

Date: December 13, 2021

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

CAUTION: Sample Buffer, Standard Diluent, 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).

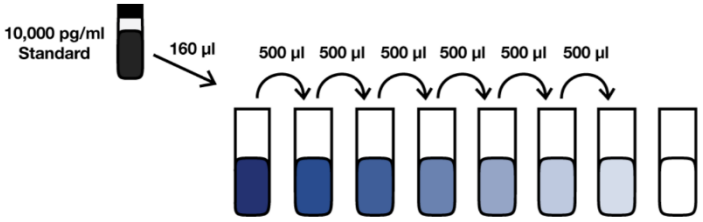
PREPARATION OF REAGENTS

Wash Solution: Wash Solution Concentrate may contain crystals; place in a warm water bath and 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. Keep at RT (22-25ºC). (**Note:** Prepare fresh Wash Buffer for each assay run.)

Cynomologus/Rhesus IFN-Alpha 2 Standard Curve Preparation:
Note: To avoid potential interference, it is recommended to run the standard curve diluted in endogenous IFN-free Sample Matrix. If Sample Matrix is unavailable, Standard Diluent may be used instead.

a. Label seven polypropylene tubes (S1 – S7).
b. Add volume of Sample Matrix (e.g. Standard Diluent, Tissue Culture Media, Serum, Plasma) to each tube as indicated in Figure 1.
c. Using polypropylene tips, add indicated amount of Cyno/Rhesus IFN-Alpha 2 to S7 and mix gently. Removed 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
Sample Matrix (µl)	840	500	500	500	500	500	500	500
IFN-Alpha (pg/ml)	1600	800	400	200	100	50	25	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 Sample Matrix. Keep at RT until use. Measurements in duplicate are recommended.

Antibody Solution: 15 minutes prior to use in step 2, dilute Antibody Concentrate in the volume of Concentrate Diluent as shown below. Keep at RT (22-25ºC).

Micro-plate Strips Used	2	4	6	8	10	12
Antibody Concentrate (µl)	20	40	60	80	100	120
Concentrate Diluent (ml)	2.0	4.0	6.0	8.0	10.0	12.0

HRP Solution: 15 minutes prior to use in step 3, dilute HRP Conjugate Concentrate in the volume of Concentrate Diluent as shown below. Keep at RT (22-25ºC).

Micro-plate Strips Used	2	4	6	8	10	12
HRP Conjugate Concentrate (µl)	10	20	30	40	50	60
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/Plate Sealers	Cyno/Rhesus IFN-Alpha 2 Standard
Wash Solution Concentrate	Antibody Concentrate
Matrices/Samples	HRP Conjugate Concentrate
Standard Diluent	
Sample Buffer	
Concentrate 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 RT (22-25ºC), 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.

Total well volume = 100 µl (Step 1 + Step 2)
Step 1: Add **50 µl** of **Sample Buffer** to every well.
Step 2: 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 1 hour.

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

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

Cover with Plate Sealer and shake at 450 rpm at RT for 1 hour.

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

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

Cover with Plate Sealer and shake at 450 rpm at RT for 1 hour.

After 1 hour, empty plate contents and wash wells four 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. DO NOT SHAKE.

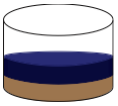
5. After 15 minutes, DO NOT EMPTY THE WELLS AND DO NOT WASH. Add **100 µl** of **Stop Solution** to each well.

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

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

CYNO/RHESUS IFN-ALPHA SERUM ELISA (46100) ASSAY PROCEDURE – QUICK REFERENCE

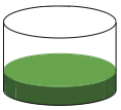
Total Time: 3 hr 15 min
Note: All incubations are at Room Temperature (RT) (22-25ºC)*



1. Add **50 µl** Sample Buffer
2. Add **50 µl** Standard, Sample or Blank

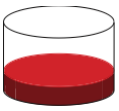
Incubate **1 hr** *(shake at 450 rpm) at RT**

Aspirate and Wash **2x**



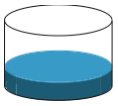
Add **100 µl** diluted Antibody Solution
Incubate ***1 hr*** *(shake at 450 rpm) at RT**

Aspirate and Wash **3x**

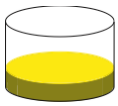


Add **100 µl** diluted HRP Solution
Incubate **1 hr** *(shake at 450 rpm) at RT**

Aspirate and Wash **4x**



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



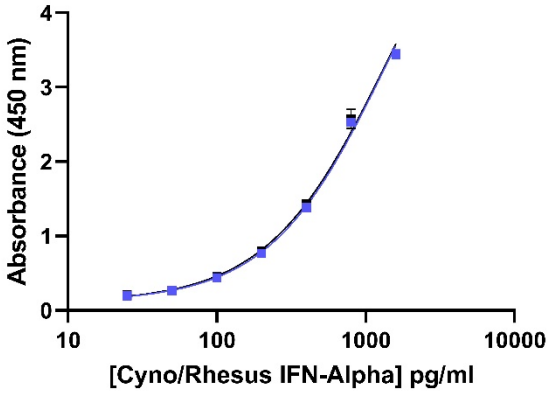
Add **100 µl** Stop Solution
Read plate within 5 min (450 nm)

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. The conversion factor of about 3-5 pg/unit is applicable for cynomologus/rhesus IFN-Alpha 2 where units are determined by comparison to human IFN-Alpha 2 international standard. Nevertheless, this conversion factor is only an approximation.

Note: The lowest limit of quantitation (LLOQ) is 25 pg/ml. Concentrations of unknown samples that measure < 25 pg/ml are suspect.

Figure 2: Typical Standard Curve



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