



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: 7584

Expiration: August 31, 2023

Store all components at 2-8°C

| Kit Components | Part No. | Lot No. | Quantity |
|--|------------|-----------|----------|
| Plate(s) | SMP135 | K7115 | 1 |
| Plate Sealers | N/A | N/A | 4 |
| Wash Solution Concentrate | SMP022-60 | K6927 | 50 ml |
| Cyno/Rhesus IFN-Alpha 2 Standard, 10,000 pg/ml | SMP141-1 | K7118 | 1 vial |
| Standard Diluent | SMP163-30 | K7119 | 25 ml |
| Sample Buffer | SMP153-8 | K7120 | 8 ml |
| Antibody Concentrate | SMP154-1 | K7121 | 1 vial |
| HRP Conjugate Concentrate | SMP056-330 | K7122 | 1 vial |
| Concentrate Diluent | SMP024-30 | K7046 | 30 ml |
| TMB Substrate Solution | KET-15 | 210201D01 | 15 ml |
| Stop Solution | SCY-15 | 59172 | 15 ml |

Product Performance Specifications

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

Authorization

Released by: _____

Date: December 20, 2022

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).

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PREPARATION OF REAGENTS

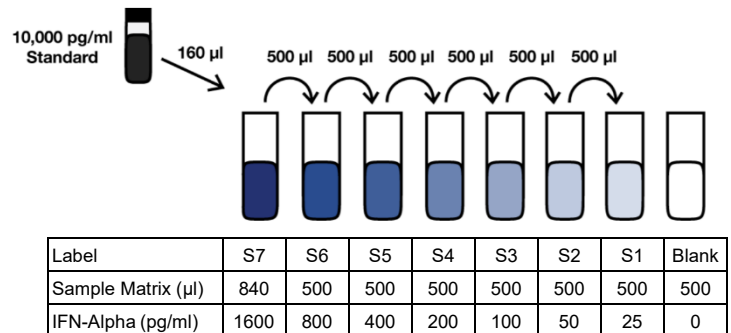
Wash Solution: Wash Solution Concentrate may contain crystals; place in a warm water bath and gently mix until completely dissolved. Prepare a 1:10 working wash solution (e.g. Add 50 ml Wash Solution Concentrate to 450 ml distilled or deionized water). Mix thoroughly before use. Keep at RT (22-25°C). (**Note:** Prepare fresh Wash Buffer for each assay run.)

Cynomolgus/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.

- Label seven polypropylene tubes (S1 – S7).
- Add volume of Sample Matrix (e.g. Standard Diluent, Tissue Culture Media, Serum, Plasma) to each tube as indicated in [Figure 1](#).
- 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



Sample Preparation: Thaw frozen sample tubes to Room Temperature (RT) (22-25°C) 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 (22-25°C).

| 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 (22-25°C).

| Micro-plate Strips Used | 2 | 4 | 6 | 8 | 10 | 12 |
|--------------------------------|-----|-----|-----|-----|------|------|
| HRP Conjugate Concentrate (µl) | 9 | 18 | 27 | 36 | 44 | 53 |
| Concentrate Diluent (ml) | 2.0 | 4.0 | 6.0 | 8.0 | 10.0 | 12.0 |

ASSAY PROCEDURE

| Bring to RT (22-25°C) | Keep at 2-8°C |
|---------------------------|----------------------------------|
| 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 (22-25°C), 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 2-8°C. 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.

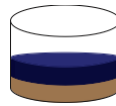
6. Using a microplate reader, determine the absorbance at 450 nm within 5 minutes after the addition of Stop Solution.

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

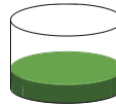
Total Time: 3 hr 15 min

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



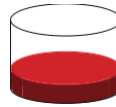
1. Add 50 µl Sample Buffer
 2. Add 50 µl Standard, Sample or Blank
- Incubate 1 hr (shake at 450 rpm) at RT*

Aspirate and Wash 2x



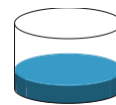
Add 100 µl diluted Antibody Solution
 Incubate 1 hr (shake at 450 rpm) at RT*

Aspirate and Wash 3x

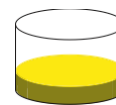


Add 100 µl diluted HRP Solution
 Incubate 1 hr (shake at 450 rpm) at RT*

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 cynomolgus/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

