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

Assay Range: 12.5 - 400 pg/ml
Compatibility: Tissue Culture Media
Assay Length: 27 hr 15 min

Catalog No: 42120-2
Lot No: 7638
Expiration: April 30, 2025
Store all components at 2-8ºC

Kit Components	Part No.	Lot No.	Quantity
Plate(s)	SMP167	K7290	5
Plate Sealers	N/A	N/A	20
Wash Solution Concentrate	SMP022-250	K7289	250 ml
Mouse IFN-Alpha Standard, 10,000 pg/ml	SMP166-2	K7284	1 vial
Sample Buffer	SMP169-250	K7288	250 ml
Antibody Concentrate	SMP170-2	K7286	1 vial
Concentrate Diluent	SMP024-60	K7227	60 ml
HRP Conjugate Concentrate	SMP179-900	K7287	1 vial
Assay Diluent	ASD-60	618856	60 ml
TMB Substrate Solution	KET-60	220103D02	60 ml
Stop Solution	SCY-60	69099	60 ml

Product Performance Specifications

	Sample Buffer	RPMI (10% FBS)*
Intra-Assay CV	≤ 8%	≤ 10%
Inter-Assay CV	≤ 10%	≤ 10%

Spike Recovery: 115% (range 82 – 135%)

* We have noticed variability in the %CV between replicates of individual points on the standard curves prepared in MEM (10% FBS). The range of the variations observed is from 0.3 - 28%. These variations do not affect the product's ability to accurately measure Mouse IFN-α independently spiked in MEM (10% FBS).

Authorization

Released by: _____

Date: July 24, 2023

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

Note: The quantitation of mouse interferon alpha in serum and plasma samples using this product has not been fully evaluated by PBL.

CAUTION: Wash Solution Concentrate, Sample Buffer and HRP/Ab Diluent contain 0.1% Kathon CG/ICP as a preservative; components should be handled with appropriate safety precautions and discarded properly. For further information, consult the safety data sheet (SDS).

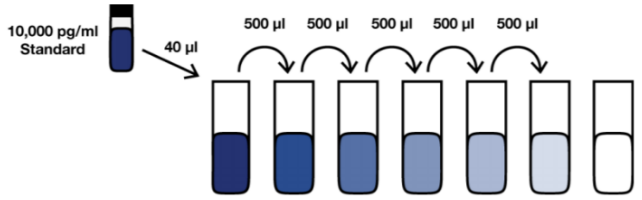
PREPARATION OF REAGENTS

Wash Solution: Wash Solution Concentrate may contain crystals; place in a warm water bath and gently mix until completely dissolved. Prepare a 1:20 working wash solution (e.g. Add 50 ml Wash Solution Concentrate to 1000 ml distilled or deionized water). Mix thoroughly before use. Keep at RT (22-25ºC). (**Note:** Prepare fresh for each assay run.)

Mouse IFN-Alpha Standard Curve Preparation:

a. Label six polypropylene tubes (S1 – S6).
b. Add volume of Sample Buffer or Sample Matrix to each tube as indicated in Figure 1. [Test samples may contain substances that can interfere with assay results, therefore it is recommended to run the IFN standard curve diluted in your Sample Matrix.]
c. Using polypropylene tips, add 40 µl Standard to S6 and mix thoroughly. Change tips between each dilution.
d. Transfer 500 µl of S6 to S5 and mix thoroughly. Repeat to complete series to S1. Set aside until use in step 1.

Figure 1: 6-Point Standard Curve Prepared in Sample Buffer



Label	S6	S5	S4	S3	S2	S1	Blank
Sample Buffer (µl)	960	500	500	500	500	500	500
IFN-Alpha (pg/ml)	400.0	200.0	100.0	50.0	25.0	12.5	0

Sample Preparation: Thaw frozen sample tubes to Room Temperature (RT) (22-25 ºC) in either tap water or between the fingertips. If samples require dilution, Sample Buffer is recommended. Keep at RT. Measurements in duplicate are recommended.

Antibody Solution: 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)	16	24	32	40	48	56
Concentrate Diluent (ml)	2.0	3.0	4.0	5.0	6.0	7.0

HRP Solution: On **Day 2**, prepare within 15 minutes prior to use. Dilute HRP Conjugate Concentrate in volume of Assay Diluent shown below. Keep at RT (22-25ºC).

Micro-plate Strips Used	2	4	6	8	10	12
HRP Conjugate Concentrate (µl)	12	20	24	32	40	48
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
Day 1	Wash Solution Concentrate	All other components
Day 2	TMB Substrate Solution	All other components
	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.
  *** Note:** Transfer plate to 2-8ºC for overnight incubation
- **Plate Washing**: All wells should be filled with a minimum of 300 µl of Wash Solution. Remove plate contents by inverting and blotting the plate on lint-free absorbent paper; tap the plate dry.

DAY 1

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 A: Add **100 µl** of **Standard**, **Test Samples** or **Blanks** (Sample Buffer or appropriate dilution matrix) to each designated well.
Step B: Add **50 µl** of diluted **Antibody Solution** to every well. Change tips between each addition.

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

After 1 hour, transfer the plate to 2-8ºC and incubate 20-24 hours. DO NOT SHAKE.

DAY 2

After 20-24 hours, empty plate contents and wash wells four times.

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

Cover with Plate Sealer and shake at 450 rpm at RT for 2 hours.

After 2 hours, empty plate contents and wash wells four times.

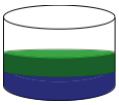
3. Add **100 µl** of **TMB Substrate Solution** to each well. Incubate **in the dark** at RT for 15 minutes. Do not use a Plate Sealer during the incubation. DO NOT SHAKE.

4. After 15 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 5 minutes after the addition of Stop Solution.

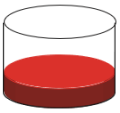
MOUSE IFN-ALPHA TCM ELISA (42120-2)
ASSAY PROCEDURE – QUICK REFERENCE

Total Time: 27 hr 15 min
Note: Unless otherwise specified, all incubations are at Room Temperature (RT) (22-25ºC)*



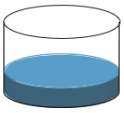
A. Add **100 µl** Standard, Sample, or Blank
B. Add **50 µl** Diluted Antibody Solution
Incubate **1 hr** *(shake at 450 rpm) at RT**
Transfer plate to 2-8ºC and incubate 20-24 hours

Aspirate and Wash **4x**

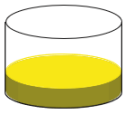


Add **100 µl** Diluted HRP Solution
Incubate **2 hr** *(shake at 450 rpm) at RT**

Aspirate and Wash **4x**



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

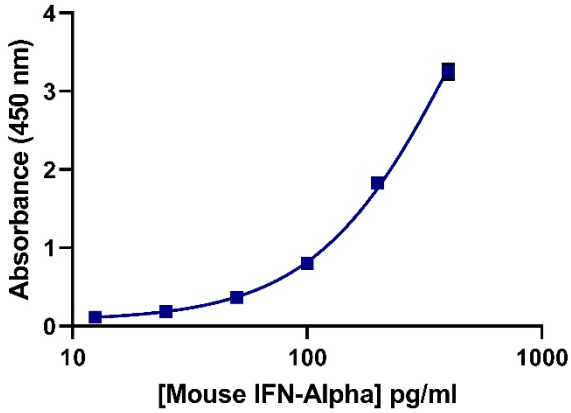


Add **100 µl** Stop Solution
Read plate within 5 min (450 nm)

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. A 4-parameter logistic plot with 1/y2 weighted analysis is recommended for obtaining optimal fit of standard curve OD values. Blank ODs may be subtracted from the standards and sample ODs to eliminate background.

Figure 2: Typical Standard Curve in Sample Diluent



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