VeriKine-HS™ Mouse Interferon Alpha All Subtype ELISA Kit
Certificate of Analysis & Protocol

Assay Range: 1.19 - 76 pg/ml, Sample Detection Range: 2.38 - 152 pg/ml
Compatibility: Serum, Plasma, Tissue Culture Media
Assay Length: 1 hr 54 min

Catalog No: 42115-1
Lot No:
Expiration:
Store all components at 2-8ºC

Kit Components	Part No.	Lot No.	Quantity
Plate(s)	SMP281		1
Plate Sealers	N/A	N/A	4
Wash Solution Concentrate	SMP022-60		2 x 50 ml
Mouse IFN-Alpha 4 Standard, 10,000 pg/ml	SMP282-1		1 vial
Sample Diluent	SMP283-30		30 ml
Antibody Concentrate	SMP284-1		1 vial
HRP Conjugate Concentrate	SMP056-120		1 vial
Antibody Diluent	SMP285-15		12 ml
HRP Diluent	ASDHRP-15		12 ml
TMB Substrate Solution	KET-15		15 ml
Stop Solution	SCY-15		15 ml

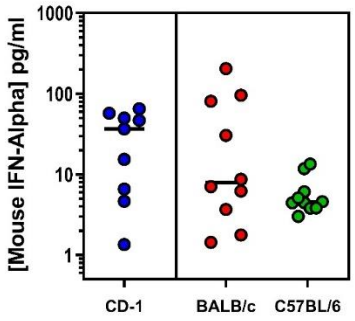
Authorization

Released by: _____

Date:

Note: Pre-screening of serum is recommended as we have determined that a significant portion of samples contain quantifiable levels of endogenous interferon alpha.

Figure 1: Endogenous Plasma and Serum Levels of Mouse IFN-Alpha



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CAUTION: Components should be handled with appropriate safety precautions and discarded properly. For further information, consult the safety data sheet (SDS).

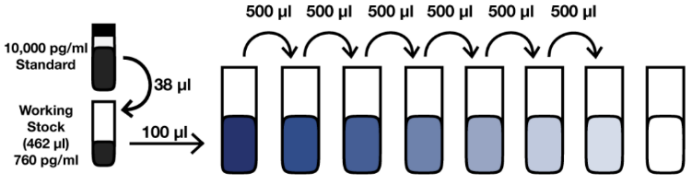
PREPARATION OF REAGENTS

Wash 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 950 ml distilled or deionized water). Mix thoroughly before use.
Note: Prepare fresh Wash Solution for each assay run.

Mouse IFN-Alpha Standard Curve Preparation:
Note 1: Sample Diluent is viscous. Pipette slowly and remove excess diluent on tip before dispensing into dilution reservoir to avoid carry over.
***Note 2:** If preparing standard curve in Sample Matrix, a 2X standard curve should be prepared and then diluted 1:2 with Sample Diluent.

a. Label seven polypropylene tubes (S1 – S7).
b. Add volume of Sample Diluent or Sample Matrix* to each tube as indicated in Figure 2.
c. Prepare *working stock* by pipetting 38 µl Standard into 462 µl Sample Diluent. Mix thoroughly by gently pipetting up and down 10 times.
d. Using polypropylene tips, add 100 µl *working stock* to S7 and mix thoroughly. **Do not change tips between each dilution.**
e. Transfer 500 µl of S7 to S6 and mix thoroughly. Repeat to complete series to S1. Set aside on ice (2-8ºC) until use in step 1.

Figure 2: 7-Point Standard Curve Prepared in Sample Diluent



Label	S7	S6	S5	S4	S3	S2	S1	Blank
Sample Diluent (µl)	900	500	500	500	500	500	500	500
IFN-Alpha (pg/ml)	76.00	38.00	19.00	9.50	4.75	2.38	1.19	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 Diluent is recommended; **for serum, plasma & media samples, a minimum 1:2 dilution is required**. Keep on ice (2-8ºC) until use. Measurements in duplicate are recommended.

Antibody Solution: Prior to starting assay, dilute Antibody Concentrate in volume of Antibody Diluent shown below. Keep on ice (2-8ºC).

Micro-plate Strips Used	2	4	6	8	10	12
Antibody Concentrate (µl)						
Antibody Diluent (ml)	1.25	2.5	3.5	5.0	6.0	7.0

HRP Solution: Prior to starting assay, dilute HRP Conjugate Concentrate in volume of HRP Diluent shown below. Keep on ice (2-8ºC)

Micro-plate Strips Used	2	4	6	8	10	12
HRP Conjugate Concentrate (µl)						
HRP Diluent (ml)	2.0	3.0	4.0	5.0	6.0	7.0

ASSAY PROCEDURE

Bring to RT (22-25ºC)	Keep at 2-8ºC
Plate/Sealers	All Other Components
Wash Solution Concentrate	
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 22-25ºC or at RT, keeping the plate away from drafts.
- **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. **Empty plate immediately after each wash. Extended soaking may lower signal.**

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** (Sample Diluent or appropriate dilution matrix) to each designated well.

Cover with Plate Sealer and shake at 650 rpm at RT for 1 hour.

After 1 hour, empty plate contents and wash wells three times.
Empty plate immediately after each wash.

2. Add **50 µl** of diluted **Antibody Solution** to each well.

Cover with Plate Sealer and shake at 650 rpm at RT for 30 minutes.

After 30 minutes, empty plate contents and wash wells three times.
Empty plate immediately after each wash.

3. Add **50 µl** of **HRP Solution** to each well.

Do not use a Plate Sealer. Shake at 650 rpm at RT for 4 minutes.
(**Note:** DO NOT allow HRP Solution to remain on plate longer than 4 minutes. We recommend removing the plate from shaker a few moments early to allow time for transport to wash station).

After 4 minutes, empty plate contents and wash wells five times.
Empty plate immediately after each wash.

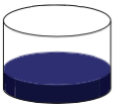
4. Add **100 µl** of **TMB Substrate Solution** to each well. Incubate **in the dark** at RT for 20 minutes. Do not use a Plate Sealer during the incubation. DO NOT SHAKE.

5. After 20 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 2 minutes after the addition of Stop Solution.

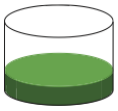
MOUSE IFN-ALPHA ALL SUBTYPE ELISA (42115)
ASSAY PROCEDURE – QUICK REFERENCE

Total Time: 1 hr 54 min



Add **100 µl** Standard, Sample, or Blank
For serum, plasma, and media samples, dilute 1:2†
Incubate **1 hr** *(shake at 650 rpm) at RT**

Aspirate and Wash **3x**

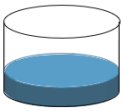


Add **50 µl** diluted Antibody Solution
Incubate **30 min** *(shake at 650 rpm) at RT**

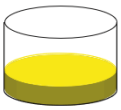
Aspirate and Wash **3x**

Add **50 µl** diluted HRP Solution
Do not seal
Incubate **4 min** *(shake at 650 rpm) at RT**

Aspirate and Wash **5x**



Add **100 µl** TMB Substrate
Incubate **20 min** *in the dark at RT**
Do not seal, shake, or wash.



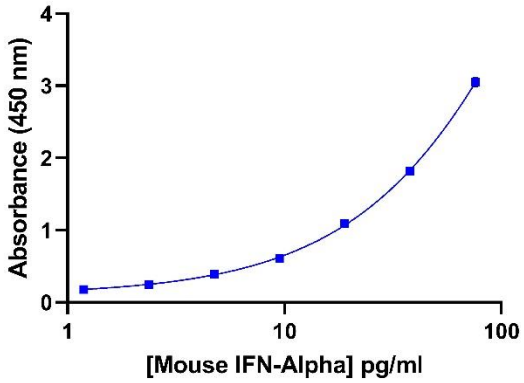
Add **100 µl** Stop Solution
Read plate within 2 min (450 nm)

†Refer to Sample Preparation on previous page.
Note: All incubations are at Room Temperature (RT) (22-25ºC)*

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. For samples that have been diluted according to the instructions given in this manual (1:2), the concentration read from the standard curve must be multiplied by the dilution factor (x2).

Figure 3: Typical Standard Curve in Sample Diluent



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