

Catalog No: 42410-2

Lot No:

Expiration:

Store all components at 2-8°C

Kit Components	Part No.	Lot No.	Quantity
Plate(s)	SMP199		5
Plate Sealers	N/A	N/A	20
Wash Solution Concentrate	SMP057-250		2 x 250 ml
Mouse IFN Beta Standard, 10,000 pg./ml	SMP195-2		1 vial
Sample Diluent	SMP196-150		150 ml
Antibody Concentrate	SMP197-2		1 vial
HRP Conjugate Concentrate	SMP056-750		1 vial
Antibody Diluent	SMP198-60		60 ml
HRP Diluent	ASDHRP-60		60 ml
Serum Buffer	SMP213-60		60 ml
TMB Substrate Solution	KET-60		60 ml
Stop Solution	SCY-60		60 ml

Authorization

Released by: _____

Date:

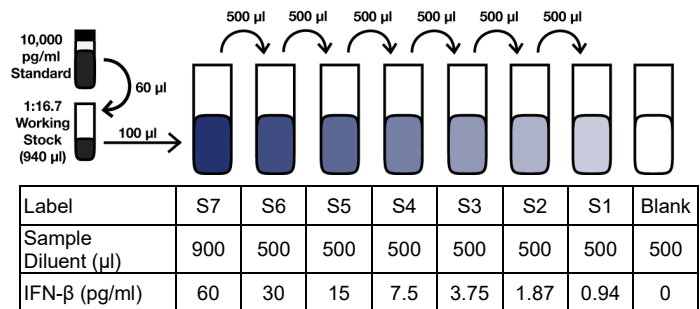
PREPARATION OF REAGENTS

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 of distilled or deionized water). Mix thoroughly before use.

Mouse IFN Beta Standard Curve Preparation:

- Prepare a 1:16.7 *working stock* of Mouse IFN- β Standard by pipetting 60 μ l of Standard into 940 μ l of Sample Diluent. Mix thoroughly by gently pipetting up and down 10 times.
- Label seven polypropylene tubes (S1 – S7).
- Add indicated volume of Sample Diluent to each tube as indicated in Figure 1.
- Add 100 μ l of *working stock* to S7 and mix thoroughly to recover all material adhered to the inside of the pipette tip.
- Using a pipette set at 500 μ l, mix S7 thoroughly. Remove indicated volume from S7 and add to S6. Mix thoroughly. Repeat to complete series to S1.
- Set aside on ice (2-8°C) until step 1.

Figure 1: 7-Point Standard Curve Prepared in Sample Diluent



Sample Preparation: Thaw frozen samples to Room Temperature (RT) (22-25°C) in either tap water or between the fingertips. If samples require dilution, prepare using Sample Diluent. Keep on ice (2-8°C) until step 1. Measurements in duplicate are recommended.

Antibody Solution: 15 minutes prior to use in step 3, dilute Antibody Concentrate in the volume of Antibody Diluent shown below. Keep on ice (2-8°C) until use.

Micro-plate Strips Used	2	4	6	8	10	12
Antibody Concentrate (μ l)						
Antibody Diluent (ml)	2.0	3.0	4.0	5.0	6.0	7.0

HRP Solution: 15 minutes prior to use in step 4, dilute HRP Concentrate in the volume of HRP Diluent shown below. Keep on ice (2-8°C) until use.

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

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

ASSAY PROCEDURE

Bring to RT (22-25°C)	Keep at 2-8°C
Plate	Mouse IFN Beta Standard
Plate Sealers	Sample Diluent
Wash Solution Concentrate	Antibody Concentrate
Serum Buffer	HRP Conjugate Concentrate
TMB Substrate Solution	Antibody Diluent
Stop Solution	HRP Diluent

- **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 250 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 2-8°C. Unused strips can be used in later assays.

2. Total well volume = 100 µl (Step A + Step B)

Step A: Add 50 µl of **Serum Buffer** (for serum or plasma samples) OR **Sample Diluent** (for tissue culture samples) to every well.

Step B: Add 50 µl of diluted **Standard, Test Samples or Blanks** (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 four times.

3. Add 50 µl of diluted **Antibody Solution** to each well. Cover with Plate Sealer and shake plate at 650 rpm at RT for 30 minutes.

After 30 minutes, empty plate contents and wash wells four times.

4. Add 50 µl of diluted **HRP Solution** to each well. Cover with Plate Sealer and shake plate at 650 rpm at RT for 10 minutes.

After 10 minutes, empty the plate contents and wash wells four times.

5. Add 100 µl of **TMB Substrate Solution** to each well. Incubate **in the dark** at RT for 10 minutes. Do not use a Plate Sealer and DO NOT SHAKE during the incubation.

6. After 10 minutes, DO NOT EMPTY THE WELLS AND DO NOT WASH. Add 100 µl of **Stop Solution** to each well.

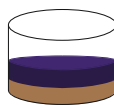
7. Using a microplate reader, determine the absorbance at 450 nm within 5 minutes after the addition of Stop Solution.

MOUSE IFN BETA SERUM ELISA (42410) ASSAY PROCEDURE – QUICK REFERENCE

Total Time: 1 hr 50 min

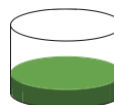
*RT: Room Temperature (22-25°C)

Note: All incubations are at RT



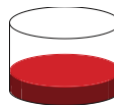
1. Add 50 µl **Serum Buffer** (for serum or plasma samples) OR **Sample Diluent** (for tissue culture samples)
2. Add 50 µl Standard, Sample or Blank
Incubate 1 hr (shake at 650 rpm) at RT*

Aspirate and Wash 4x



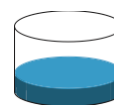
- Add 50 µl diluted **Antibody Solution**
Incubate 30 min (shake at 650 rpm) at RT*

Aspirate and Wash 4x

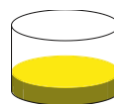


- Add 50 µl diluted **HRP Solution**
Incubate 10 min (shake at 650 rpm) at RT*

Aspirate and Wash 4x



- Add 100 µl **TMB Substrate Solution**
Incubate 10 min in the dark at RT*
Do not seal, shake or wash.

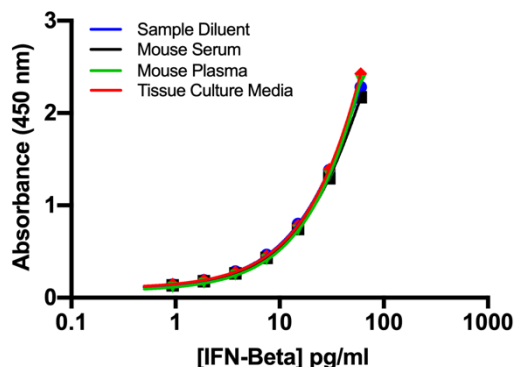


- Add 100 µl **Stop Solution**
Read plate within 5 min (450 nm)

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. Blank ODs may be subtracted from the standards and sample ODs to eliminate background.

Figure 2: Typical Standard Curves in Various Matrices



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