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**Mouse Interferon Beta ELISA Kit**  
 v.1.2

Product #42400-2  
 Five Plate (480 Tests)

**Assay Range:** 15.6 – 1000 pg/ml

**Incubation Time:** 3 hr 15 min

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To ensure proper kit performance, please review the protocol in its entirety prior to running the assay. Please note that concentrations of the Antibody Concentrate and HRP Conjugate Concentrate vary from lot to lot as a result of calibrating each kit for optimal sensitivity. Refer to the lot specific Certificate of Analysis (COA), provided with the kit, for final Antibody Concentrate and HRP Conjugate Concentrate dilutions. Only components included with the kit should be used in the final assay to ensure accurate results.

**Specificity:** Mouse IFN- $\beta$ . *No cross reactivity with human IFN- $\alpha$ , IFN- $\gamma$ , IFN- $\kappa$ , IFN- $\beta$ ; Rat IFN- $\alpha$ , IFN- $\gamma$ , IFN- $\beta$ ; Mouse IFN- $\alpha$ , IFN- $\gamma$ ; Feline IFN- $\alpha$ ; or Pig IFN- $\alpha$*

**Specifications:** This kit quantitates mouse interferon beta in serum and media using a sandwich enzyme immunoassay.<sup>1, 2</sup> This ELISA kit utilizes Tetramethyl-benzidine (TMB) as the substrate. All reagents to properly run the assay are supplied, including pre-coated microtiter plates (96 wells). Typical standard curves for each kit lot are provided within the Certificate of Analysis.

**Special Conditions/Comments:** For retention of activity, all reagents should be stored at 2-8°C in the dark **unless stated otherwise**. Deionized or distilled water should be used for preparation of all reagents. All dilutions should be made with

polypropylene tubes and pipette tips. Pipette tips should be changed between each dilution tube. All measurements for standards and samples should be performed in duplicate. At least two control wells (with Sample Diluent only) should be used for each assay; these control values should be subtracted from all readings prior to any calculations or plots of the data.

**\*\*Caution\*\*** Solutions A, C, and F contain 0.1% Kathon CG/ICP as a preservative; they should be handled with appropriate safety precautions and discarded properly. For further information, consult the material safety data sheet for Kathon CG/ICP.

**Materials Supplied:**

Kit Components	#42400-2
<b>Pre-Coated Microtiter Plate</b> <i>(12 1x8 well strips)</i>	5
<b>Plate Sealers</b>	20
<b>A: 10x Wash Concentrate</b>	250 ml
<b>B: Mouse IFN-<math>\beta</math> Standard</b> <b>500,000 pg/ml</b> <i>(stock solution)</i>	150 $\mu$ l
<b>C: Sample Diluent</b>	150 ml
<b>D: Antibody Concentrate</b>	1 vial
<b>E: HRP Conjugate Concentrate</b>	1 vial
<b>F: Concentrate Diluent</b>	150 ml
<b>G: TMB Substrate Solution</b>	60 ml
<b>H: Stop Solution</b>	60 ml

**Additional Materials Required** *(but not provided):*

1. Microtiter plate reader capable of reading at a wavelength of 450 nm.
2. Room temperature (22-25°C) incubator.
3. Variable volume microliter pipettes capable of accurately delivering 10  $\mu$ l, 90  $\mu$ l, and 100  $\mu$ l.
4. Adjustable multichannel pipette (50-200 $\mu$ l) for dispensing of reagents.
5. Reagent reservoirs for multichannel pipettes (polypropylene).
6. Wash bottle or plate washing system.
7. Distilled or deionized water.
8. Serological pipettes (1, 5, 10 or 25 ml).
9. Disposable pipette tips (polypropylene).
10. Timer with alarm capable of measuring to an accuracy of +/- 1 second.
11. 1000 ml graduated cylinder.

**Storage Conditions:**

<b>Unopened Kit</b>	<b>Store at 2-8°C.</b> Do not use past the expiration date of the kit lot.
<b>Diluted Wash Buffer</b>	<b>Store at 2-8°C.</b> Do not use past the expiration date of the kit lot.
<b>Sample Diluent</b> <i>(Bottle C)</i>	
<b>Concentrate Diluent</b> <i>(Bottle F)</i>	
<b>Stock Mouse IFN Standard</b> <i>(Vial B)</i>	
<b>Antibody Concentrate</b> <i>(Vial D)</i>	
<b>HRP Conjugate Concentrate</b> <i>(Vial E)</i>	
<b>Microtiter Plate</b>	

**Procedure:**

1. Prepare Wash Buffer as follows: Dilute the 50 ml of 10x wash concentrate (Bottle A) with 450 ml of distilled or deionized water. The Final wash solution should be stored at 2-8°C and mixed thoroughly before use. All the wash steps should be performed at room temperature (22-25°C). Wash steps can be performed manually with a squirt bottle or by using an automated plate washer.
2. Prepare a 1:10 working stock of the Mouse IFN Standard by pipetting 10  $\mu$ l of the Mouse IFN- $\beta$  Standard (Vial B) in 90  $\mu$ l of sample diluent (Bottle C). From this working stock, **prepare the standard curve using the amounts in the table shown below**. Samples of unknown interferon concentration to be tested should also be diluted in the sample diluent as required. Prepare fresh dilutions of the standard curve for each assay run.

**Note:** *In certain situations “test” samples may contain substances that can interfere with assay results. Therefore, it may be beneficial to run the IFN standard curve diluted in your sample matrix (e.g. normal mouse serum) in addition to the sample diluent provided.*

**Preparation of Standard Curve:**

Tube No.	working stock	S <sub>7</sub>	S <sub>6</sub>	S <sub>5</sub>	S <sub>4</sub>	S <sub>3</sub>	S <sub>2</sub>	S <sub>1</sub>	BL
<b>Amount Taken from Tube to Left (ml)</b>	---	0.020	0.5	0.5	0.5	0.5	0.5	0.5	---
<b>Sample Diluent (ml)</b>	---	0.98	0.5	0.5	0.5	0.5	0.5	0.5	1.0
<b>Final Conc. (pg/ml)</b>	50000	1000	500	250	125	62.5	31.3	15.6	0

- Determine the number of microplate wells required to test the desired number of samples and standard curve. Place precisely 100 µl of the interferon samples and standard curve prepared in Step 2 in individual wells of the microtiter plate, at least in duplicate. Remaining strips should be resealed with desiccant in the foil pouch and stored at 2-8°C for future use.

A recommended microplate layout for the standard curve concentrations (S<sub>1</sub>-S<sub>7</sub>) and blanks (BL) is shown in the diagram below. Samples of unknown interferon concentration should be tested as required.

	1	2	3	4	5	6	7	8	9	10	11	12
A	BL	BL										
B	S <sub>1</sub>	S <sub>1</sub>										
C	S <sub>2</sub>	S <sub>2</sub>										
D	S <sub>3</sub>	S <sub>3</sub>										
E	S <sub>4</sub>	S <sub>4</sub>										
F	S <sub>5</sub>	S <sub>5</sub>										
G	S <sub>6</sub>	S <sub>6</sub>										
H	S <sub>7</sub>	S <sub>7</sub>										

BL: Blank (Sample diluent only) S<sub>1</sub> – S<sub>7</sub>: IFN-β standard curve

- Cover the microtiter plate with one of the enclosed plastic plate sealers and incubate for 60 min in a closed chamber at 22-25°C. Optionally, the incubations can be carried out at room temperature, keeping the plate away from drafts and other temperature fluctuations. After the 60 min incubation, wash three times (3x) with prepared wash solution (at least 250 µl/well). Washes can be performed manually with a squirt bottle or by utilizing an automated plate washer. Blot well after the final wash.
- During the incubation period, prepare the Antibody Solution for use in step 6. Dilute the Antibody Concentrate with Concentrate Diluent (Bottle F). Refer to the lot specific Certificate of Analysis (COA) for the correct amounts of Antibody Solution to prepare. Store used undiluted antibody concentrate at 2-8°C for future use.
- Add 100 µl of the **Antibody Solution** prepared using the **COA**, to each well. Cover the microtiter plate with one of the enclosed plastic plate sealers and incubate for 60 min in a closed chamber at 22-25°C. Optionally, the incubations can be carried out at room temperature, keeping the plate away from drafts and other temperature fluctuations. After the 60 min incubation, wash three times (3x) with prepared wash solution (at least 250 µl /well). Washes can be

performed manually with a squirt bottle or by utilizing an automated plate washer. Blot well after the final wash.

- During the incubation period, prepare the HRP solution for use in step 8. Dilute the HRP Conjugate Concentrate with HRP Conjugate Diluent. Refer to the lot specific Certificate of Analysis (COA) for the correct amounts of HRP Solution to prepare. Store unused undiluted HRP concentrate at 2-8°C for future use.
- Add 100 µl of the **HRP solution** prepared using the **COA**, to each well. Cover the microtiter plate with one of the enclosed plastic plate sealers and incubate for 60 min in a closed chamber at 22-25°C. Optionally, the incubations can be carried out at room temperature, keeping the plate away from drafts and other temperature fluctuations. After the 60 min incubation, wash three times (3x) with prepared wash solution (at least 250 µl/well). Washes can be performed manually with a squirt bottle or by utilizing an automated plate washer. Blot well after the final wash.

**Note: During the HRP incubation period, warm the TMB Substrate Solution (Bottle G) to room temperature.**

- Add 100 µl of the TMB Substrate Solution (Bottle G) to each well. Incubate for 15 min in a closed chamber at 22-25°C. Optionally, the incubations can be carried out at room temperature, keeping the plate away from drafts and other temperature fluctuations in the dark.

**Note: Do not use the plate sealer at this step.**

- Add 100 µl of Stop Solution (Bottle H) to each well. Mix gently. Use caution to avoid bubbles as this can affect results.
- Using a microplate reader, determine the absorbance at 450 nm within 5 minutes after the addition of the stop solution.
- Unknown sample concentrations can be determined by extrapolation off the standard curve. Typical standard curves for this assay are depicted on the lot specific Certificate of Analysis.

### Procedure: *At a Glance*

Step	Volume per well	Incubation	No. of Washes
Blank/Standard/Sample	100 µl	60 min RT	3x
2° Antibody	100 µl	60 min RT	3x
HRP	100 µl	60 min RT	3x
TMB	100 µl	15 min RT	---
STOP	100 µl	---	---
<b>Read at 450 nm</b>			

### References

- Staehelin, T., Stähli, C., Hobbs, D.S., and Pestka, S. (1981) "A Rapid Quantitative Assay of High Sensitivity for Human Leukocyte Interferon with Monoclonal Antibodies," in *Methods in Enzymology*, Vol. 79 (S. Pestka, ed.), Academic Press, New York, 589-595.
- Balachandran, S., Thomas, E., and Barber, G. (2004) "A FADD-dependent innate immune mechanism in mammalian cells", in *Nature*, Vol 432, 401- 405