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**Mouse Interferon Gamma**  
**(Mu-IFN- $\gamma$ ) ELISA Kit**  
 v. 1.4

Product #42500-1 Single Plate (96 Tests)

Lot Number:

Expiration Date:

**High Sensitivity Protocol:** 25 – 1000 pg/ml

**Extended Range Protocol:** 125 – 5000 pg/ml

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**Please review the protocol in its entirety prior to use to insure proper kit performance. Please note that the concentrations of the Detecting Antibody and HRP differ from lot to lot as a result of calibrating each kit for optimal sensitivity.**

**Speed:** Incubation time, 3 hr 15 min

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

**Specifications:** This kit quantitates mouse interferon gamma in media using a sandwich immunoassay.<sup>1,2</sup> The kit is based on an ELISA with anti-secondary antibody conjugated to horseradish peroxidase (HRP). Tetramethyl-benzidine (TMB) is the substrate. All reagents are supplied. One pre-coated microtiter plate (96 wells) is included. The assay is based on the international reference standard for mouse interferon gamma (Mu-IFN- $\gamma$ ) provided by the National Institutes of Health.<sup>3</sup> Typical standard curves for each lot are included with the procedure.

**Special Conditions/Comments:** For retention of activity, all reagents should be kept at 2-6°C in the dark. 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 (wells with Dilution Buffer 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, B, C, D and F contain 0.1 g/L thimerosal as a preservative; they should be handled with appropriate safety precautions and discarded properly. Since thimerosal is highly toxic through skin contact, inhalation or ingestion, suitable protective wear and care should be used in handling these solutions. For further information, consult the material safety data sheets for thimerosal (CAS #54-64-8).

**Materials Supplied:**

Kit Components	#42500-1	
Size: single plate	Lot#	Volume
Plate (s)		1
Plate Sealers	n/a	4
A: Wash Solution Concentrate		50 ml
B: Mouse IFN Gamma Solution 100,000pg/mL		55 $\mu$ l
C: Dilution Buffer		50 ml
D: Antibody Concentrate		1 vial
E: HRP Conjugate Concentrate		1 vial
F: HRP Conjugate Diluent		15 ml
G: TMB Substrate Solution		15 ml
H: Stop Solution		15 ml

**Procedure:**

- Before starting assays, prepare Final Wash Solution as follows. Dilute 50 ml of the Wash Solution Concentrate (Bottle A) to 1000 ml with distilled water. The Final Wash Solution should be stored in the refrigerator and mixed thoroughly before use. All the wash steps should be performed at room temperature (24°C) with ice cold (2 – 6° C) wash solution.
- Construct a standard curve from 0 – 1000 pg/ml (High Sensitivity) or 0 – 5000 pg/ml (Extended Range) by serial dilutions of the Mouse Interferon Gamma Solution (Vial B) in the Dilution Buffer (Bottle C). **Please note that the sample curves provided are for reference only.**

Label seven polypropylene tubes and serially dilute the Mouse Interferon Gamma Solution as shown below. All dilutions should be made with polypropylene tubes and pipette tips. Pipette tips should be changed between each dilution. Samples of unknown interferon concentration to be tested should also be diluted in the Dilution Buffer as required.

**High Sensitivity**

Tube No.	Vial B	S <sub>6</sub>	S <sub>5</sub>	S <sub>4</sub>	S <sub>3</sub>	S <sub>2</sub>	S <sub>1</sub>	B
Amount Taken from Tube to Left (ml)	---	0.01	0.5	0.4	0.5	0.5	0.5	---
Dilution Buffer Bottle C (ml)	---	0.99	0.5	0.6	0.5	0.5	0.5	1.0
Final Conc. (pg/ml)	100,000	1000	500	200	100	50	25	0

**Extended Range**

Tube No.	Vial B	S <sub>6</sub>	S <sub>5</sub>	S <sub>4</sub>	S <sub>3</sub>	S <sub>2</sub>	S <sub>1</sub>	B
Amount Taken from Tube to Left (ml)	---	0.025	0.25	0.25	0.2	0.25	0.25	---
Dilution Buffer Bottle C (ml)	---	0.475	0.25	0.25	0.3	0.25	0.25	0.5
Final Conc. (pg/ml)	100,000	5000	2500	1250	500	250	125	0

- Determine the number of microplate wells required to test the desired number of samples. We recommend the use of 16 wells for blanks (BK) and standards (S<sub>1</sub>-S<sub>6</sub>). Remove extra microtiter strips from the frame, seal in the foil bag provided and store at 2 – 6 °C. Unused strips can be used in later assays.
- Place precisely 100  $\mu$ l of the interferon samples prepared in Step 2 in individual wells of the microtiter plate, at least in duplicate.

	1	2	3	4	5	6	7	8	9	10	11	12
A	BK	BK										
B	BK	BK										
C	S1	S1										
D	S2	S2										
E	S3	S3										
F	S4	S4										
G	S5	S5										
H	S6	S6										

BK: Blank – buffer only

S<sub>1</sub> – S<sub>6</sub> : serially diluted standards

A recommended microplate sample layout for blanks and standards is shown in the diagram above. Samples of unknown interferon concentration should be tested as required.

5. Cover the microtiter plate with one of the enclosed plastic plate sealers and incubate for 1 hour in a closed chamber at 24°C. Optionally, the incubations can be carried out at room temperature, keeping the plate away from drafts and other temperature fluctuations.

6. During the incubation period, prepare the Antibody Solution for use in step 8. To avoid loss of material, centrifuge the Antibody Concentrate (Vial D) for a few seconds to bring the liquid to the bottom of the vial. For each microplate strip used, add  $\mu\text{l}$  of diluted Antibody Concentrate to 1 ml Bottle C, Dilution Buffer (High Sensitivity) or  $\mu\text{l}$  Antibody Concentrate to 1 ml Dilution Buffer (Extended Range). See the table below for sample dilutions.

Microplate strips used		2	4	6	8	10	12
Antibody Concentrate Vial D	High Sensitivity ( $\mu\text{l}$ )						
	Extended Range ( $\mu\text{l}$ )						
Dilution Buffer Bottle C		2ml	4ml	6ml	8ml	10ml	12ml

7. After the first incubation, empty the contents of the plate and wash the wells one time only with the Final Wash Solution (see Step 1). Each well should be filled when washing. It is best to use a free-flowing washer attached to a reservoir, such as the Nunc Immunowash, as opposed to a manual pipettor. After washing, invert and blot the plate on lint-free absorbent paper. Tap the plate dry. If hazardous samples are used, proper precautions should be taken.

8. After washing, add 100  $\mu\text{l}$  of the Antibody Solution (see Step 6) to each well. Cover the plate with a plate sealer and incubate for 1 hour at 24°C.

*NOTE: For stability reasons Vial E is provided as a concentrate and must be pre-diluted prior to use, proceed to step 9 for further instruction. Please do not attempt to measure the volume in the vial prior to dilution as it may affect kit performance. Use the diluted material from Step 9 to prepare the working solutions in Step 10.*

9. During the incubation period, prepare the HRP Conjugate Concentrate (Vial E) for use in step 10. To avoid loss of material, centrifuge the HRP Conjugate Concentrate for a few seconds to bring the liquid to the bottom of the vial. Add 105  $\mu\text{l}$  of the HRP Conjugate Diluent (Bottle F) to the vial and mix gently, centrifuge again if necessary.

10. Prepare the following working solutions of HRP as follows.

**High Sensitivity:** For each microstrip used add  $\mu\text{l}$  of HRP Conjugate Concentrate (from step 9) to 1.0 ml Bottle F.

**Extended Range:** For each microstrip used add  $\mu\text{l}$  of HRP Conjugate Concentrate (from step 9) to 1.0 ml Bottle F.

See table below for sample dilutions

Microplate strips used	2	4	6	8	10	12
Diluted HRP Conjugate Vial E (step 9)	High Sensitivity ( $\mu\text{l}$ )					
	Extended Range ( $\mu\text{l}$ )					
HRP Conjugate Diluent Bottle F	2ml	4ml	6ml	8ml	10ml	12ml

Aliquot unused HRP Conjugate Concentrate (Vial E) in appropriate portions and store at  $-70^{\circ}\text{C}$  until use.

10. After the incubation, empty the contents of the plate and wash the wells three times with the Final Wash Solution as in Step 7. After washing, invert and blot the plate on lint-free absorbent paper. Tap the plate to dry. If hazardous samples are used, proper precautions should be taken.

11. After washing, add 100  $\mu\text{l}$  of HRP Conjugate Solution (see Step 9) to each well. Cover the plate with a plate sealer and incubate for 1 hour at 24°C.

12. During the incubation period, warm the TMB Substrate Solution (Bottle G) to 24°C.

13. After the incubation, empty the contents of the plate and wash the wells four times with the Final Wash Solution as in Step 7. After washing, invert and blot the plate on lint-free absorbent paper. Tap the plate to dry. If hazardous samples are used, proper precautions should be taken.

14. After washing, add 100  $\mu\text{l}$  of TMB Substrate Solution (Bottle G) to each well. Cover the plate with a plate sealer and incubate for 15 minutes at 24°C in the dark.

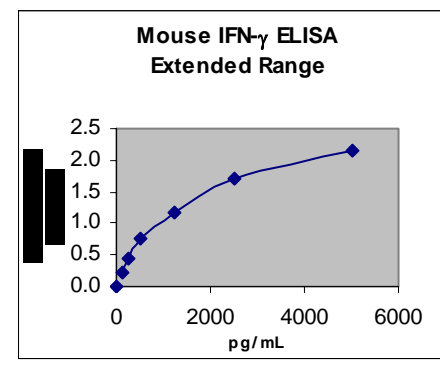
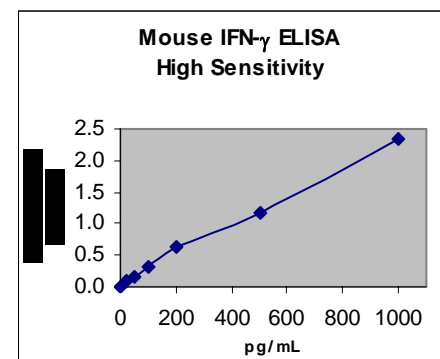
15. After the incubation, add 100  $\mu\text{l}$  of Stop Solution (Bottle H) to each well. Mix by swirling or tapping the plate gently.

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

17. By graphing the data for the standard curve, the interferon titer in the samples can be determined by comparison. Typical standard curves for this assay are shown on the enclosed pages.

*Because the interferon samples are titrated against the international standard, the values from the curves can be determined in units/ml as well as pg/ml. The conversion factor of about 100 pg/unit is applicable for mouse interferon gamma. Nevertheless, this conversion factor is only an approximation.*

The following standard curve for Mouse IFN $\gamma$  ELISA is provided as a demonstration only and should not be used to obtain test results. A standard curve must be run for each set of samples assayed.



#### References

1. 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.
2. Kelder, B., Rashidbaigi, A., and Pestka, S. (1986) "A Sandwich Radioimmunoassay for Human IFN- $\gamma$ ," in Methods in Enzymology, Vol. 119 (S. Pestka, ed.), Academic Press, New York, 582-587.
3. Mouse IFN- $\gamma$  international reference standard provided by the NIH, reference no. Gxa02-901-533. Pestka, S. (1986) "Interferon Standards and General Abbreviations," in Methods in Enzymology, Vol. 119 (S. Pestka, ed.), Academic Press, New York, 14-23.

