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Human Interferon Gamma (Hu-IFN-γ) ELISA Kit

v.1.4

Product #41500-1
Single Plate (96 Tests)

Lot Number

Expiration Date:

Sensitivity: 12.5 – 500 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: Human IFN-γ. No cross reactivity with human IFN-α or human IFN-β. No cross-reactivity with: mouse or rat IFN-α, IFN-β, or IFN-γ.

Specifications: This kit quantitates human interferon gamma in media using a sandwich immunoassay.[1,2] 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 human interferon gamma (Hu-IFN-γ) provided by the National Institutes of Health.[3] 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	#41500-1	
Size: single plate	**Lot #**	**Volume**
Plate (s)		1
Plate Sealers	n/a	4
A: Wash Solution Concentrate		50 ml
B: Human IFN Gamma Solution 10,000pg/ml		0.2 ml
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:

1. 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 preformed at room temperature (24°C) with ice cold (2 – 6° C) wash solution.

2. Construct a standard curve from 0 – 500 pg/ml by serial dilutions of the Human Interferon Gamma Solution (Vial B) in the Dilution Buffer (Bottle C). To avoid loss of material, centrifuge the Human Interferon Gamma solution for a few seconds to bring the liquid down to the bottom of the vial. ***Please note that the sample curves provided are for reference only.***

   Label seven polypropylene tubes and serially dilute the Human 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.

Tube No.	*Vial B*	*S_6*	*S_5*	*S_4*	*S_3*	*S_2*	*S_1*	*B*
Amount Taken from Tube to Left (ml)	---	0.05	0.4	0.5	0.5	0.5	0.5	---
Dilution Buffer Bottle C (ml)	---	0.95	0.6	0.5	0.5	0.5	0.5	1.0
Final Conc. (pg/ml)	*10,000*	*500*	*200*	*100*	*50*	*25*	*12.5*	*0*

3. 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_1-S_6). Remove extra microtiter strips from the frame, <u>seal</u> in the foil bag provided and store at 2 – 6 °C. Unused strips can be used in later assays.

4. Place precisely 100 µl 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_1 – S_6: 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 µl Antibody Concentrate to 1 ml Bottle C, Dilution Buffer.
See the table below for sample dilutions.

Microplate strips used	*2*	*4*	*6*	*8*	*10*	*12*
Antibody Concentrate Vial D (µl)						
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). It is best to use a free-flowing washer attached to a reservoir, such as the Nunc Immunowash, as opposed to a manual pipettor. Each well should be filled when washing. 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 µl 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 9A for further instruction. Please do not attempt to measure the volume in the vial prior to dilution as it may affect kit performance.

9A. During the incubation period, prepare the HRP Conjugate Concentrate (Vial E) for use in step 9B. 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 105µl of the HRP Conjugate Diluent (Bottle F) to the vial and mix gently, centrifuge again if necessary.

9B. For each microplate strip used add µl of HRP Conjugate Concentrate diluted in step 9A to 1.0 ml of Bottle F, HRP Conjugate Diluent. Refer to table for sample dilutions.

Microplate Strips Used	*2*	*4*	*6*	*8*	*10*	*12*
Pre-diluted HRP Conjugate (Step 9A) (µl)						
HRP Conjugate Diluent Bottle F	*2ml*	*4ml*	*6ml*	*8ml*	*10ml*	*12ml*

Aliquot unused HRP Conjugate Concentrate (Vial E) diluted in step 9A and store at –70°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 µl of HRP Conjugate Solution (see Step 9B) 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 µl 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.

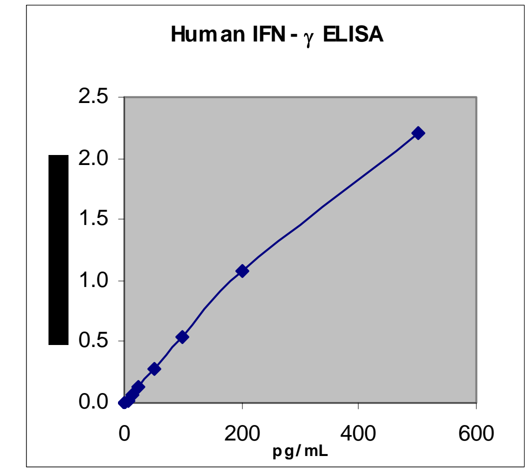
15. After the incubation, add 100 µl of Stop Solution (Bottle H) to each well. Mix by swirling or tapping the plate gently.

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 human interferon gamma.[4] Nevertheless, this conversion factor is only an approximation.

The following standard curve for Human IFN γ 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-γ," in Methods in Enzymology, Vol. 119 (S. Pestka, ed.), Academic Press, New York, 582-587.

3. Human IFN-γ international reference standard provided by the NIH, reference no. Gg23-901-530. Pestka, S. (1986) "Interferon Standards and General Abbreviations," in Methods in Enzymology, Vol. 119 (S. Pestka, ed.), Academic Press, New York, 14-23.

4. Kung, H. –F., Pan, Y.-C., Moschera, J., Tsai, K., Bekesi, E., Chang, M., Sugino, H., and Honda, S. (1986). "Purification of Recombinant Human Immune Interferon," *Methods in Enzymology*, Vol. 119 (S. Pestka, ed), Academic Press, New York, 204-210.