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Protocol

VeriKine™ Human Interferon Gamma ELISA Kit

Catalog No: 41500
Lot No: 7172
Expiration: January 31, 2021
Assay Range: 12.5 - 500 pg/ml
Store all components at 2 – 8°C

Please review the protocol in its entirety prior to use to ensure 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.

Materials Provided:

- Pre-coated microtiter plate
- Plate sealers
- Wash Solution Concentrate
- Human Interferon Gamma Standard, 10,000 pg/ml
- Dilution Buffer
- Antibody Concentrate
- HRP Conjugate Concentrate
- Assay Diluent
- TMB Substrate Solution
- Stop Solution

Additional Materials Required (NOT PROVIDED):

- Microtiter plate reader capable of reading an OD at a wavelength of 450 nm
- Variable volume microtiter pipettes
- Adjustable multichannel pipette (50-300 µl)
- Reagent reservoirs
- Wash bottle or plate washing system
- Distilled or deionized water
- Serological pipettes (1, 5, 10 or 25 ml)
- Disposable pipette tips (polypropylene)

Kit Components	Part No.	Lot No.	Quantity
Plate	SMP034	T1383	1
Plate Sealers	N/A	N/A	4
Wash Solution Concentrate	SMP164-60	T1386	50 ml
Human IFN-γ Standard, 10,000 pg/ml	SMP035-1	T1387	0.2 ml
Dilution Buffer	SMP010-15	T1388	15 ml
Antibody Concentrate	SMP036-1	T1389	1 vial
HRP Conjugate Concentrate	JAC-2	134096	1 vial
Assay Diluent	ASD-30	361866	30 ml
TMB Substrate Solution	KET-15	180803D02	15 ml
Stop Solution	SCY-15	51066	15 ml

Specifications: This kit quantitates human interferon gamma in media using a sandwich immunoassay.[1,2] The kit is based on an ELISA with anti-detection 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.

Speed: Incubation time, 3 hr 15 min

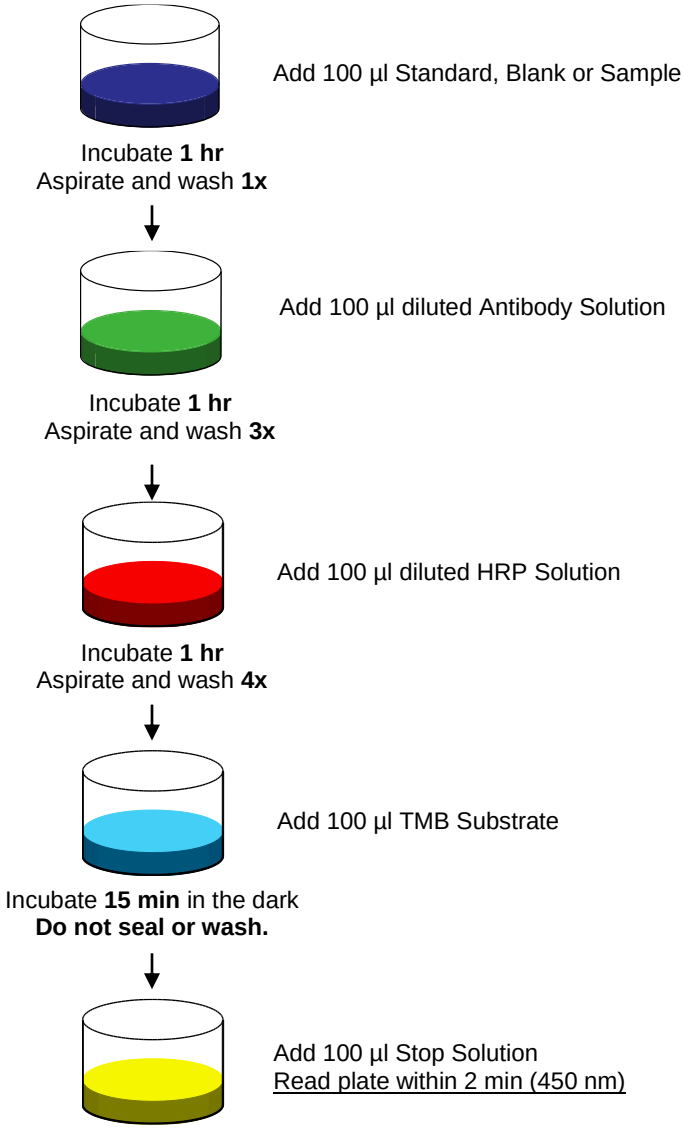
Specificity: Human IFN-γ. No cross reactivity detected with human IFN-α or human IFN-β. No cross reactivity detected with mouse or rat IFN-α, IFN-β or IFN-γ.

Storage Conditions/Comments: For retention of full activity, all reagents should be kept at 2-8°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: The Wash Solution Concentrate, Human IFN Gamma Solution, Dilution Buffer and Antibody Concentrate 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 (MSDS) for thimerosal (CAS #54-64-8).

Assay Procedure – Quick Reference

Total Time: 3 hr 15 min



Note: All incubations are at room temperature (22-25°C).

Preparation of Reagents

Before starting the assay, the plate, Wash Solution Concentrate, applicable dilution matrices, Dilution Buffer, Assay Diluent, Stop Solution and samples should be equilibrated to room temperature (RT), 22-25°C. The TMB Substrate Solution should be equilibrated to RT (22-25°C) during step 3 of the Assay Procedure. Supplied Human IFN Gamma Standard, Antibody Concentrate and HRP Conjugate Concentrate should be kept on ice (4°C).

Wash Solution: The Wash Solution Concentrate may contain crystals. Place the bottle in a warm water bath and gently mix until completely dissolved. Prepare a 1:10 working wash solution by adding 50 ml of Wash Solution Concentrate to 450 ml of distilled or deionized water. Mix thoroughly before use. Diluted Wash Solution can be stored at RT (22-25°C) when not in use. All the wash steps should be performed at RT (22-25°C).

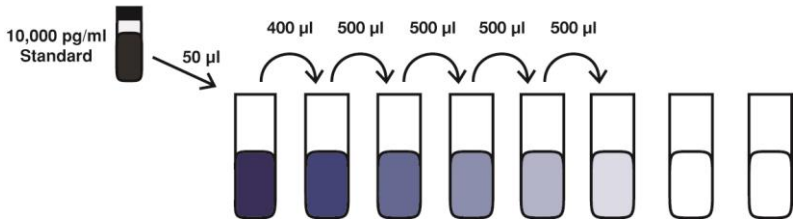
Human Interferon Gamma Solution: Dilute the Human Interferon Gamma Standard, provided at 10,000 pg/ml, in the Dilution Buffer as indicated below. To avoid loss of material, centrifuge the Human Interferon Gamma solution for a few seconds to bring the liquid down to the bottom of the vial.

In certain situations "test" samples may contain substances that can interfere with assay results. Therefore, it is recommended to run the INF standard curve diluted in your sample matrix.

Standard Curve Preparation:
Construct a standard curve 0-500 pg/ml in the Dilution Buffer.

a) Label six polypropylene tubes as S1-S6.
b) Fill tubes with Dilution Buffer as indicated.
c) Using polypropylene tips, add the Human IFN Gamma Standard to S6 and mix gently. Change tips between each dilution.
d) Remove indicated amount from S6 and add to S5. Repeat to complete series to S1.

Figure 1: 6-Point Standard Curve Prepared in Dilution Buffer



Label	S6	S5	S4	S3	S2	S1	Blank	Blank
Dilution Buffer or Sample Matrix Vol. (µl)	950	600	500	500	500	500	500	500
IFN-γ Conc. (pg/ml)	500	200	100	50	25	12.5	0	0

Test Sample Preparation: Prepare test samples of unknown interferon concentration to be tested using Dilution Buffer as required. Measurements in duplicate are recommended.

Antibody Solution: To avoid loss of material, centrifuge the Antibody Concentrate for a few seconds to bring the liquid to the bottom of the vial. For each microtiter strip used, add 10 µl Antibody Concentrate to 1 ml Assay Diluent. Refer to table for sample dilutions. Prepare 15 minutes prior to use in step 2 of the Assay Procedure and keep at RT (22-25°C).

Microtiter strips used	2	4	6	8	10	12
Antibody Concentrate (µl)	20	40	60	80	100	120
Assay Diluent	2 ml	4 ml	6 ml	8 ml	10 ml	12 ml

HRP Solution: To avoid loss of material, centrifuge the HRP Conjugate Concentrate for a few seconds to bring the liquid to the bottom of the vial.

a. Add 245 µl of Assay Diluent to the vial and mix gently, centrifuge again if necessary.
b. For each microtiter strip used, add 5 µl HRP Conjugate Concentrate to 1 ml Assay Diluent. Refer to table for sample dilutions.

Prepare 15 minutes prior to use in step 3 of the Assay Procedure and keep at RT (22-25°C). Aliquot unused HRP Conjugate Concentrate (Step "a") diluted in Assay Diluent and store at -70°C until use.

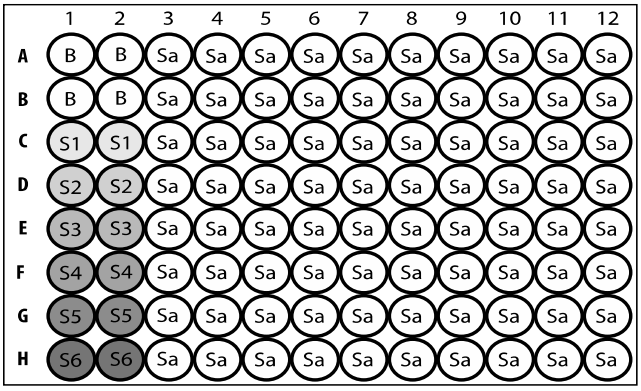
Microtiter strips used	2	4	6	8	10	12
Pre-diluted HRP Conjugate (Step a) (µl)	10	20	30	40	50	60
Assay Diluent	2 ml	4 ml	6 ml	8 ml	10 ml	12 ml

NOTE: For stability reasons HRP Conjugate Concentrate is provided as a concentrate and must be pre-diluted prior to use. Please do not attempt to measure the volume in the vial prior to dilution as it may affect kit performance.

Assay Procedure

All incubations should be performed at room temperature (RT), 22-25°C, keeping the plate away from drafts and other temperature fluctuations. Use plate sealers to cover the plate as directed. During all wash steps remove contents of plate by inverting and shaking over a sink and blotting the plate on lint-free absorbent paper; tap the plate. All wells should be filled with at least 250 µl of diluted Wash Solution during each wash step. Refer to Preparation of Reagents for dilution of concentrated solutions.

Figure 2: Example of a Typical Plate Setup



B = Blank
S1 – S6 = Standard Curve
Sa = Sample

1. **Standards and Test Samples:** Each standard, blank and sample should be run at least in duplicate. Determine the number of microtiter plate strips required to test the desired number of samples plus the appropriate number of wells needed to run blanks and standards. We recommend using strips 1 and 2, rows A-H, for serially diluted standards and blanks. Remove extra microtiter plate strips from the frame, seal in the foil bag provided and store at 2-8°C. Unused strips can be used in later assays.

   Add 100 µl of prepared interferon standards, samples or blanks to individual wells of the microtiter plate. Cover with plate sealer and incubate plate at RT (22-25°C) for 1 hour.

   After 1 hour, empty the contents of the plate and wash the wells one time with diluted Wash Solution.

2. **Antibody Solution:** Add 100 µl of diluted Antibody Solution to each well. Cover with plate sealer and incubate at RT (22-25°C) for 1 hour.

   After 1 hour, empty the contents of the plate and wash the wells three times with diluted Wash Solution.

3. **HRP Solution:** Add 100 µl of diluted HRP Solution to each well. Cover with plate sealer and incubate at RT (22-25°C) for 1 hour. During this incubation period, warm the TMB Substrate Solution to RT (22-25°C).

   After 1 hour, empty the contents of the plate and wash the wells four times with diluted Wash Solution.

4. **TMB Substrate:** Add 100 µl of the TMB Substrate Solution to each well. Incubate, in the dark, at RT (22-25°C), for 15 minutes. DO NOT USE a plate sealer during the incubation.

5. **Stop Solution:** After the 15 minute incubation of TMB, DO NOT EMPTY THE WELLS AND DO NOT WASH. Add 100 µl of Stop Solution to each well.

6. **Read:** Using a microplate reader, determine the absorbance at 450 nm within 2 minutes after the addition of the Stop Solution.

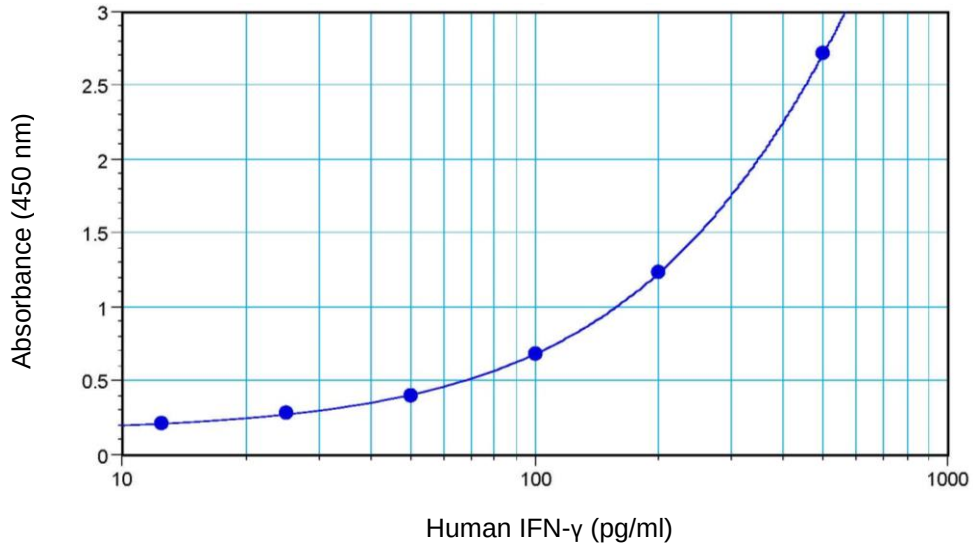
Calculation of Results

By plotting by optical densities (OD) using a 4-parameter fit for the standard curve, the interferon titer in the samples can be determined. Blank ODs should be subtracted from the standards and sample ODs to eliminate background.

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 VeriKine™ Human Interferon Gamma ELISA is provided as demonstration only and should not be used to obtain test results. A standard curve must be run for each set of samples assayed.

Figure 3: Typical Standard Curve

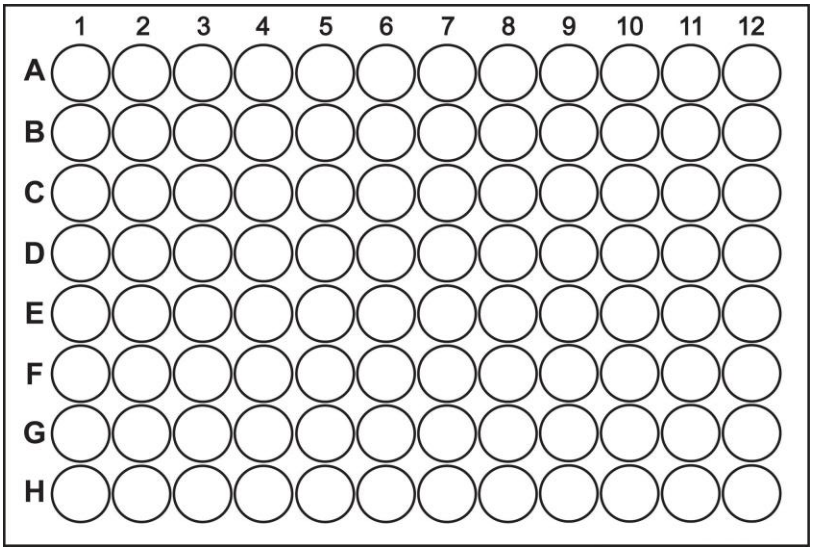


References

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) Human IFN-Gamma international reference standard provided by the NIH, reference no. Gg23-901-530. Pestka, S. (1986) "Interferons 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 /hojnda, S. (1986) "Purification of Recombinant Human Immune Interferon," *Methods in Enzymology*, Vol. 119 (S. Pestka, ed.), Academic Press, New York, 204-210.

Plate Layout

Use this plate layout as a record of standards and samples assayed.



Authorization

Released by: _____ Date: January 16, 2020