Human IFN-γ ELISA Kit
(CAT NO: abs510007)

For quantitative detection of human IFN-γ concentrations in cell culture supernatants, serum and plasma samples.

This package insert must be read in its entirety before using this product.
For research use only. Not for use in diagnostic procedures.

IMD (Hong Kong)

Address: Unit 513, 5/F, Biotech Centre 2, No. 11 Science Park West Avenue, Hong Kong Science Park, Sha Tin, Hong Kong

Website: www.immunodiagnostics.com.hk

Email: info@immunodiagnostics.com.hk

Tel: (+852) 3502 2780

IMD (Canada)

Address: 3330 Bayview Avenue, Block #6, Toronto, M2M 3R8, Ontario, Canada

Email: info@immunodiagnostics.ca

Tel: +1-437-886-5136

BACKGROUND

Interferon-gamma (IFN-γ, also known as Type II interferon) is a crucial cytokine with immunomodulatory functions. IFN-γ signaling is via IFN-γ RI (the alpha subunit) and IFN-γ RII. IFN-γ plays a key role in host defense through its antiviral, antiproliferative, and immunomodulatory functions. IFN-γ can induce the production of cytokines and upregulate the expression of different membrane proteins, including MHC-I/II (major histocompatibility complex), Fc receptors, leukocyte adhesion molecules, and B7 family antigens in various types of cells. IFN-γ effectively activates macrophages, guides B cell immunoglobulin synthesis, class switching, and secretion. It also influences the development of T helper cell subtypes by inhibiting Th2 differentiation and stimulating Th1 growth.

ASSAY PRINCIPLE

This assay kit employs the double antibody sandwich enzyme-linked immunosorbent assay (ELISA) technique. Specific anti-human IFN-γ antibodies with high affinity are pre-coated onto the enzyme-linked plate. In the wells of the enzyme-linked plate, standards, test samples, and biotinylated detection antibodies are added. After incubation, IFN-γ present in the samples binds to the solid-phase antibody and the detection antibody, forming an immune complex. After removing unbound substances, streptavidin-horseradish peroxidase labeled with horseradish peroxidase (HRP) is added. After wash step to remove any unbound substances, 3,3′,5,5′-Tetramethylbenzidine (TMB)substrate is added and color develops in proportion to the amount of IFN-γ bound initially. The assay is stopped, and the optical density of the wells is determined using a micro-plate reader at 450nm wavelength (with a reference wavelength of 540nm or 570nm) for quantification.

REAGENTS SUPPLIED

Item	Description	Format	Storage Of Opened / Reconstituted Material
Human IFN-γ Microplate	96 well polystyrene microplates coated with a monoclonal antibody specific for Human IFN-γ	1 plate	Return the unused strips to the aluminum foil bag with the desiccant bag, seal it tightly. May be stored for up to 30 days at 2-8 °C.
Human IFN-γ Standard	Recombinant Human IFN-γ α in a buffered protein base with preservatives; lyophilized. Refer to the vial label for reconstitution volume.	2 vials	Once dissolved, aliquot and store at -20°C for up to 14 days. Avoid repeated freeze-thaw cycles.
Human IFN-γ Detection Antibody	Lyophilized powder	1 vial	Once dissolved, store at 2-8°C for 14 days.
40×SA-HRP	40×Streptavidin-HRP	1 vial (0.3mL)	Store at 40× concentration at 2-8°C; it is not recommended to store at 1× working concentration.
10×Reagent Diluent	10×Reagent Diluent, please dilute with distilled water to 1× before use.	1 vial (6mL)	After opening, store at 2-8°C for up to 30 days.
Substrate Solution	TMB Substrate	1 vial (11mL)	
Stop Solution	Strong acid, used to disrupt the HRP enzyme structure.	1 vial (6mL)	
25×Wash Buffer	25× concentrated solution of buffered surfactant with preservative.	1 vial (20mL)	

Plate Sealer	96-well plate sealing film	3 pieces	Store at room temperature, do not reuse to avoid contamination.

OTHER MATERIALS REQUIRED, BUT NOT PROVIDED

1. Pipettes and pipette tips.
2. Beakers, flasks, cylinders necessary for preparation of reagents.
3. Buffer and reagent reservoirs.
4. Paper towels or absorbent paper.
5. Plate reader capable of reading absorbency at 450 nm, 540 nm or 570 nm.
6. Distilled water or deionized water.
7. Statistical calculator with program to perform regression analysis.

STORAGE

1) The kit should be stored at 2-8°C, and all reagents should be equilibrated to room temperature before use. Immediately after use remaining reagents should be returned to cold storage (2-8°C).
2) Expiry of the kit and reagents is stand on labels.
3) Once opened, the strips may be stored at 2-8°C for up to one month.

SAMPLE COLLECTION AND STORAGE INSTRUCTIONS

The sample collection and storage conditions listed below are intended as general guidelines. Sample stability has not been evaluated.

Cell Culture supernatants: Remove particulates by centrifugation. Test the sample immediately or aliquot and store at ≤ -20°C. Avoid repeated freeze-thaw cycles.

Serum: Collect samples using serum separation tubes (SST) and allow samples to clot at room temperature for 30 minutes. Centrifuge for 15 minutes at a speed of 1000 g. Collect serum and assay immediately or aliquot and store them at ≤-20°C. Avoid repeated freeze-thaw cycles.

Plasma: Collect plasma using EDTA, heparin, or citrate as anticoagulants. Centrifuge for 15 minutes at 1000 g within 30 minutes of collection. Assay immediately or aliquot and store them at ≤-20°C to avoid repeated freeze-thaw cycles.

Note: The sample may need to be diluted with 1×Reagent Diluent.

PREPARATION OF REAGENTS

Bring all reagents to room temperature for 15 minutes before use.

1. 1×Wash buffer:

Prepare 1×Wash buffer by mixing the 25×Wash buffer (20 ml) with 480 ml of distilled water or deionized water. The 1×Wash buffer may be stored at 2-8°C for up to one month.

2. Reagent Diluent:

Prepare 1×Reagent Diluent by mixing the 10×Reagent Diluent (6 ml) with 54 ml of distilled water or deionized water. The 1×Reagent Diluent may be stored at 2-8°C for up to one month.

3. Detection Antibody Working Solution:

Centrifuge the lyophilized powder to the bottom of the tube and dissolve it in 110 µL of 1× Reagent Diluent to make a 100× Detection Antibody Stock Solution. Allow it stand at room temperature for 5 minutes. Dilute the 100× Detection Antibody Stock Solution with 1× Reagent Diluent to create 1×Detection Antibody Working Solution.

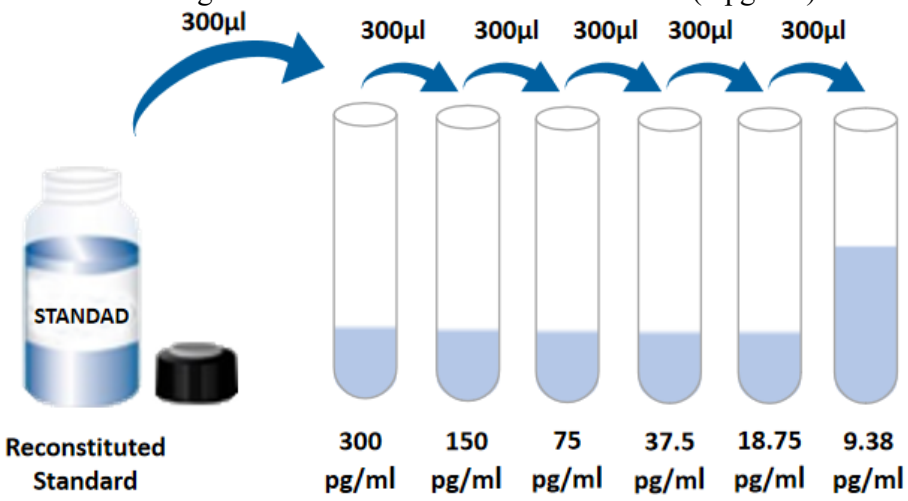
100 µL of 1×Detection antibody solution is required per well. Prepare only as much 1×Detection solution as needed. Return the 100×Detection antibody solution to 2-8°C immediately after the necessary volume is pipetted.

4. 1×SA-HRP Working Solution:

The SA-HRP in this kit is a 40× stock solution. Please dilute with 1×Reagent Diluent to 1×SA-HRP Working Solution before use. 100 µL of the 1×SA-HRP Working Solution is required per well.

5. Reconstituted Standard:

Centrifuge the standard tube briefly before opening the cap. Add 1000 µL 1×Reagent Diluent into lyophilized standard to generate the first standard (600 pg/ml). Pipette 300 µL of 1×Reagent Diluent into each tube. Use the Reconstituted Standard to produce a dilution series (below). Mix each tube thoroughly before the next transfer. 1×Reagent Diluent serves as the zero standard (0 pg/mL).



ASSAY PROCEDURE

It is recommended that all samples be assayed in duplicate.

1. Prepare reagents, samples, and standard dilutions as directed by the previous sections.
2. Equilibrate the microplate to room temperature. Return excess strips to the foil pouch containing the desiccant pack and reseal.
3. Add 100 µL of standards of different concentrations or samples per well. Cover with the Plate Sealer provided. Mix by gently tapping the plate frame for 1 minute. Incubate for 2 hours at room temperature.
4. Aspirate each well and wash with 300 µL 1×Wash Buffer. Repeating the process three times. Complete removal of liquid at each step is essential to good performance. After the last wash, remove any remaining 1× Wash Buffer by aspirating or inverting the plate and tap the plate on clean paper towels.
5. Add 100 µL of 1× Detection Antibody Working Solution to each well. Cover with a new Plate Sealer. Incubate for 2 hours at room temperature.
6. Repeat the aspiration/wash as in step 5.
7. Add 100 µL of 1×SA-HRP Working Solution to each well. Incubate for 20 minutes at room temperature. Protect from light.
8. Repeat the aspiration/wash as in step 5.
9. Add 100 µL of Substrate Solution to each well. Incubate for 5-30 minutes at room temperature. Protect from light.
10. Add 50 µL of Stop Solution to each well. Gently tap the plate to ensure thorough mixing. (The color of the solution in the well will change from blue to yellow)
11. Determine the optical density of each well within 30 minutes, using a microplate reader set to 450 nm. If wavelength correction is available, set to 540 nm or 570 nm. If wavelength correction is not available, subtract readings at 540 nm or 570 nm from the readings at 450 nm. This subtraction will correct for optical imperfections in the plate. Readings made directly at 450 nm without correction may be higher and less accurate.

CALCULATION OF RESULTS

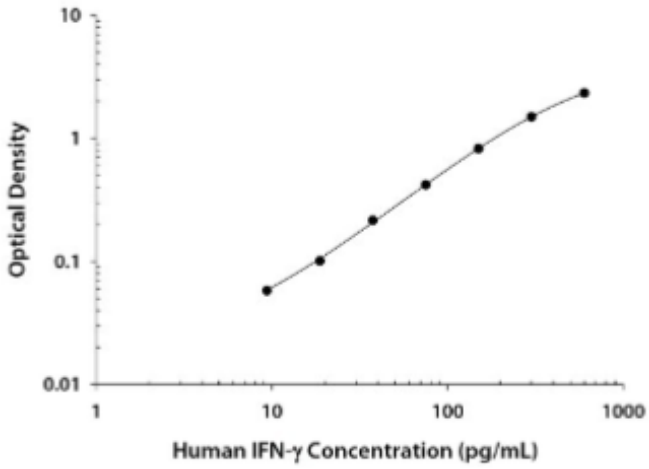
1. Average the duplicate readings for each standard, control, and sample and subtract the average zero standard optical density (O.D.).
2. Create a standard curve by reducing the data using computer software capable of generating a four-parameter logistic (4-PL) curve-fit. As an alternative, construct a standard curve by plotting the mean absorbance for each standard on the y-axis against the concentration on the x-axis and draw a best

fit curve through the points on the graph. The data may be linearized by plotting the log of the concentrations versus the log of the O.D. and the best fit line can be determined by regression analysis. This procedure will produce an adequate but less precise fit of the data.

3. If samples have been diluted, the concentration read from the standard curve must be multiplied by the dilution factor.

TYPICAL DATA

This standard curve is provided for demonstration only. A standard curve should be generated for each set of samples assayed.



ASSAY CHARACTERISTICS

Recovery

Human IFN- γ of different levels was spiked into cell culture medium samples to determine its recovery rate. The recovery rate ranged from 90-116%, with an average recovery rate of 103%.

Sensitivity

The minimum detectable dose (MDD) of Human IFN-γ is typically less than 7.8 pg/mL.
The MDD was determined by adding two standard deviations to the mean O.D. value of twenty zero standard replicates and calculating the corresponding concentration.

Linearity

To assess the linearity of the assay, four samples spiked with high concentrations of Human IFN-γ were diluted with 1×Reagent Diluent to bring them within the detection range.

Dilution	Recovery (%)
1:1	107
1:2	111
1:4	111
1:8	106

Specificity

This assay recognizes natural and recombinant Human IFN-γ.
The factors listed below were prepared at 50 ng/mL in 1×Reagent Diluent and assayed for cross-reactivity.
Preparations of the following factors prepared at 50 ng/mL in a mid-range Human IFN-γ control were assayed for interference. No significant cross-reactivity or interference was observed.

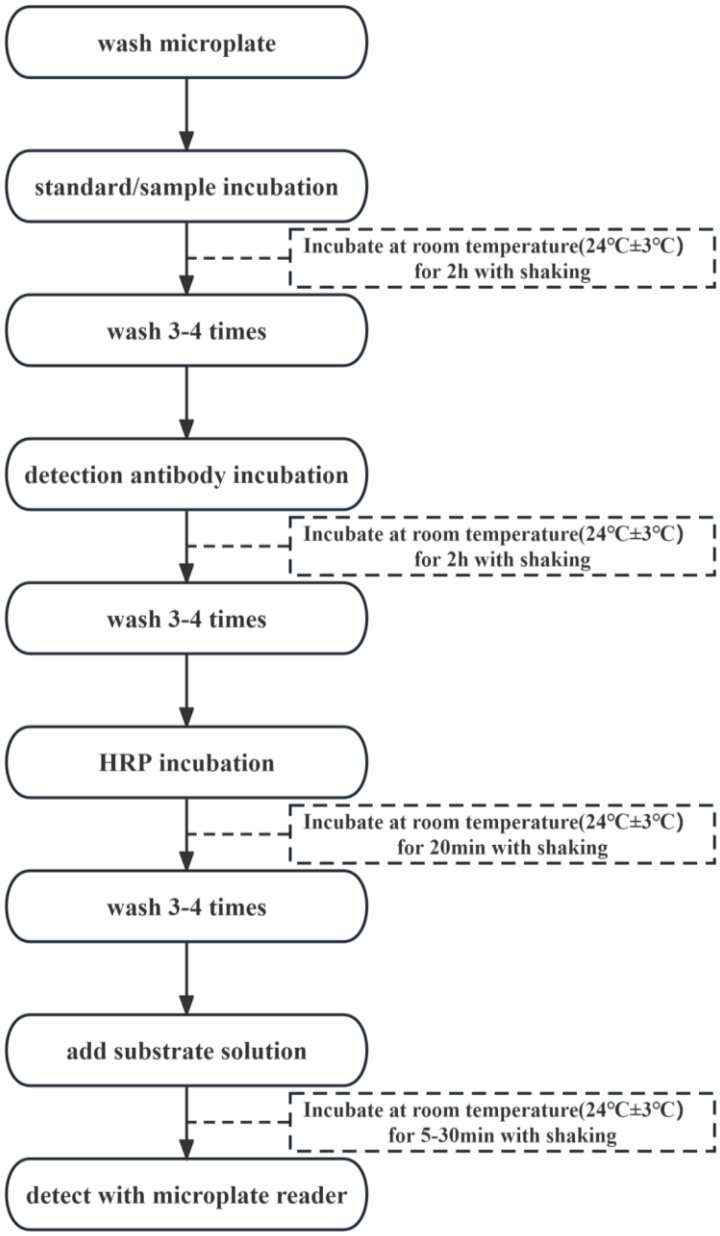
Recombinant human protein	Recombinant IFN-γ protein from other species
IFN-β; IFN-γR1	Bovine IFN-γ Mouse IFN-γ Canine IFN-γ Porcine IFN-γ

TROUBLESHOOTING

1. Weak or no signal in ELISA

Possible Cause	**Solution**
Incorrect storage of components. Mix or substitute reagents with those from other lots or sources.	Purchase a new kit, please pay attention to the storage conditions. Do not mix or substitute reagents with those from other lots or sources.
Incorrect assay temperature (too cold). Incubation times inadequate.	Bring substrates to room temperature before use. Extend the incubation time.
Reagents added incorrectly	Check protocol, ensure reagents were added in the proper order and prepared to correct dilution.
The container used for preparing the solution is not clean, or water used incorrectly.	Use a clean container and qualified distilled water.
Improper washing of the plate.	Use an automated plate washer, if available. Wash according to the instructions in the manual.
Antibody or HRP concentration is too low.	Use according to the instruction manual, and do not dilute arbitrarily.

2. Too much signal in ELISA

Possible Cause	Solution
Insufficient washing	Use appropriate washing procedure. At the end of each washing step, invert plate on absorbent towels and allow to completely drain, tapping forcefully if necessary to remove any residual fluid.
The chromogenic substrate (TMB) is contaminated or exposed to metal ions or oxidants.	Dilute reagents using a clean container and qualified distilled water. Store in the dark.
Incubation time too long. Incorrect temperature.	Control time and temperature for incubation and enzyme-catalyzed reactions.
Cross-contamination caused by not changing the pipette tips.	A new pipette tip should be used for each sample.
Cross-contamination between adjacent wells.	Tap the plate vertically and use appropriate plate paper to prevent paper debris or dust from entering the wells.
The sample contains endogenous interfering substances.	Predict possible interfering substances and deal with them accordingly.
Sample hemolysis, long storage, incomplete agglutination, bacterial contamination, and the influence of additives in blood collection tubes.	Avoid hemolysis, contamination, and prolonged storage.

SUMMARY OF ASSAY PROCEDURE