# Human IFN-gamma Sandwich ELISA Kit Datasheet

Please read it entirely before use

**Catalogue Number:** KE00781

**Size:** 96T

**Sensitivity:** 1.1 pg/mL

**Range:** 7.8-500 pg/mL

**Usage:** For the quantitative detection of human IFN-gamma concentrations in serum, plasma and cell culture supernatant.

**This product is for research use only and not for use in human or animal therapeutic or diagnostic.**



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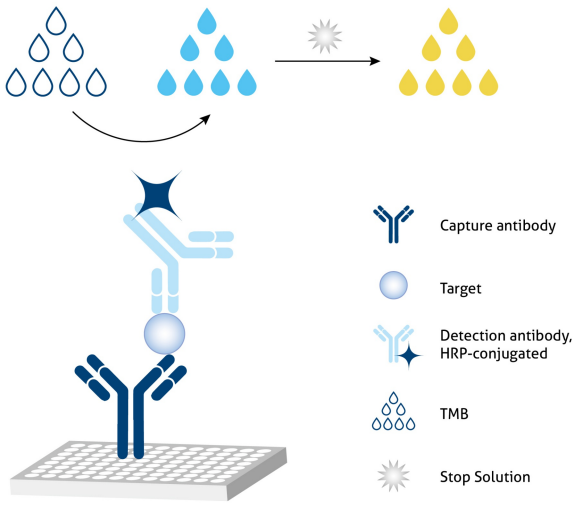
## 1. Background

Interferon gamma (IFNG) is a soluble cytokine that is the only member of the type II class of interferons. It is secreted by Th1 cells, cytotoxic T cells and NK cells. The cytokine is associated with antiviral, immunoregulatory and anti-tumor properties and is a potent activator of macrophages. It plays a crucial role in pathogen clearance. Aberrant IFNG expression is associated with a number of autoinflammatory and autoimmune diseases. It has been identified in many studies as a biomarker for pleural tuberculosis (TB). Mutations in this gene are associated with aplastic anemia.

## 2. Principle




**Sandwich ELISA structure (Detection antibody labeled with HRP)**

A capture antibody is pre-coated onto the bottom of wells which binds to analyte of interest. A detection antibody labeled with HRP also binds to the analyte. TMB acts as the HRP substrate and the solution color will change from colorless to blue. A stop solution containing sulfuric acid turns solution yellow. The color intensity is proportional to the quantity of bound protein which is measurable at 450 nm with the correction wavelength set at 630 nm.

## 3. Required Materials

3.1 A microplate reader capable of measuring absorbance at 450 nm with the correction wavelength set at 630 nm.

3.2 Calibrated, adjustable precision pipettes and disposable plastic tips. A manifold multi-channel pipette is recommended for large assays.

3.3 Plate washer: automated or manual.

3.4 Absorbent paper towels.

3.5 Glass or plastic tubes to prepare standard and sample dilutions.

3.6 Beakers and graduated cylinders.

3.7 Log-log or semi-log graph paper or computer and software for ELISA data analysis. A four-parameter logistic (4-PL) curve-fit is recommended.



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## 4. Kit Components and Storage

| | | |
|---|---|---|
| **Microplate** - antibody coated 96-well microplate (8 well × 12 strips) | 1 plate | **Unopened Kit:** Store at 2-8°C for 6 months or -20°C for 12 months. **Opened Kit:** All reagents stored at 2-8°C for 7 days. **Please use a new standard for each assay.** |
| **Protein standard** - 1000 pg/bottle; lyophilized | 2 bottles | |
| **Detection antibody, HRP-conjugated (100×)** - 120 µL/vial* | 1 vial | |
| **Additional Diluent AT-00781** - 6 mL/bottle. Only for serum and plasma samples. | 1 bottle | |
| **Sample Diluent PT 4B1** - 30 mL/bottle | 2 bottles | |
| **Detection Diluent** - 30 mL/bottle | 1 bottle | |
| **Wash Buffer Concentrate (20×)** - 30 mL/bottle | 1 bottle | |
| **Tetramethylbenzidine Substrate (TMB)** - 12 mL/bottle | 1 bottle | |
| **Stop Solution** - 12 mL/bottle | 1 bottle | |
| **Plate Cover Seals** | 4 pieces | |

* Centrifugation immediately before use

## 5. Safety Notes

5.1 Avoid any skin and eye contact with Stop Solution and TMB. In case of contact, wash thoroughly with water.

5.2 Do not use the kit after the expiration date.

5.3 Do not mix or substitute reagents or materials from other kit lots or other sources.

5.4 Be sure to wear protective equipment such as gloves, masks and goggles during the experiment.

5.5 When using an automated plate washer, adding a 30 second soak period following the addition of Wash Buffer to improve assay precision

## 6. Sample Collection and Storage

6.1 Serum: Allow blood samples to clot for 30 minutes, followed by centrifugation for 15 minutes at 1000xg. Clear serum can be assayed immediately or aliquoted and stored at -20°C. Avoid repeated freeze-thaw cycles.

6.2 Plasma: Use EDTA, heparin, or citrate as an anticoagulant for plasma collection. Centrifuge for 15 minutes at 1000xg within 30 minutes of collection. The plasma can be assayed immediately or aliquoted and stored at -20°C. Avoid repeated freeze-thaw cycles.

6.3 Cell Culture Supernatant: Remove particulates by centrifugation for 5 minutes at 500xg and assay immediately or aliquot and store samples at ≤ -20°C. Avoid repeated freeze-thaw cycles.



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## 7. Regent Preparation

**7.1 Wash Buffer (1X):** If crystals have formed in the concentrate, warm to room temperature and mix gently until the crystals have completely dissolved. Add 30 mL of Wash Buffer Concentrate(20X) to 570 mL deionized or distilled water to prepare 1X Wash Buffer.

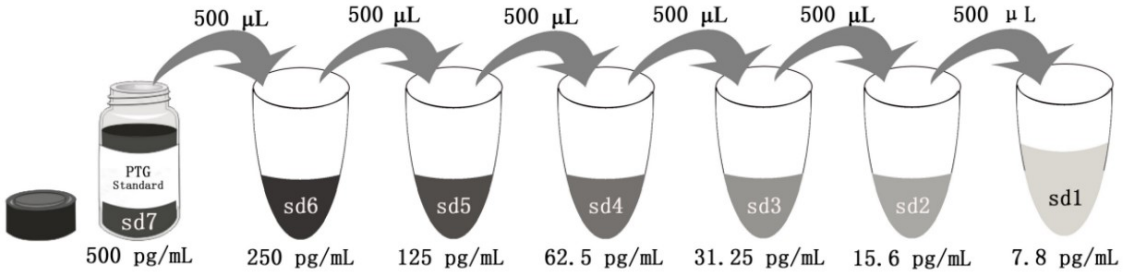
**7.2 Detection Antibody, HRP-conjugated(1X):** Dilute 100X Detection Antibody, HRP-conjugated 1:100 using Detection Diluent prior to assay. Suggested 1:100 dilution: 10 µL 100X Detection Antibody, HRP-conjugated + 990 µL Detection Diluent (Centrifuge the 100X Detection Antibody solution, HRP-conjugated for a few seconds prior to use).

**7.3 Sample Dilution:** Different samples should be diluted with corresponding Sample Diluent, samples may require further dilution if the readout values are higher than the highest standard OD reading. Variations in sample collection, processing and storage may affect the results of the measurement.

Recommended Dilution for different sample types: 1:2 is recommended for human serum and plasma; 1:2 to 1:600 is recommended for cell culture supernatant.

**7.4 Standard Serial Dilution:**

Add 2 mL Sample Diluent PT 4B1 in protein standard.




| Add # µL of Standard diluted in the previous step | — | 500 µL | 500 µL | 500 µL | 500 µL | 500 µL | 500 µL |
|---|---|---|---|---|---|---|---|
| # µL of Sample Diluent PT 4B1 | **2000 µL** | 500 µL | 500 µL | 500 µL | 500 µL | 500 µL | 500 µL |
| | "sd7" | "sd6" | "sd5" | "sd4" | "sd3" | "sd2" | "sd1" |



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## 8. Assay Procedure Summary

**Bring all reagents to room temperature before use (Detection antibody, HRP-conjugated can be used immediately). To avoid cross-contamination, change pipette tips between additions of each standard level, between sample additions, and between reagent additions. Also, use separate reservoirs for each reagent.**

8.1 Take out the required number of microplate strips and return excess strips to the foil pouch containing the drying reagent pack and reseal; store at 4°C immediately. Microplate strips should be used in one week.

8.2 Preset the layout of the microplate, including control group,standard group and sample group;

For serum or plasma, add 50 µL of Additional Diluent to the appropriate wells (No need incubation and wash);

For cell culture supernatant, no need to add Additional Diluent, directly follow the next step.

8.3. Add 100 µL of each standard and sample to the appropriate wells.(Make sure sample addition is uninterrupted and completed within 5 to 10 minutes).

8.4 Seal plate with cover seal, pressing it firmly onto top of microwells. Incubate the plate for 2 hours at 37°C.

8.5 Wash

1) Gently remove the cover seal. Discard the liquid from wells by aspirating or decanting. Remove any residual solution by tapping the plate a few times on fresh paper towels.

2) Wash 4 times with 1X Wash Buffer, using at least 350-400 µL per well. Following the last wash, firmly tap plates on fresh towels 10 times to remove residual Wash Buffer. Avoid getting any towel fibers in the wells or wells drying out completely.

8.6 Add 100 µL of 1X Detection antibody, HRP-conjugated solution (refer to Reagent Preparation7.2) to each well. Seal plate with cover seal and incubate for 40 minutes at 37°C.

8.7 Repeat wash step in 8.5.

8.8 Signal development: Add 100 µL of TMB substrate solution to each well, protected from light. Incubate for 15 to 20 minutes. Substrate Solution should remain colorless until added to the plate.

8.9 Quenching color development: Add 100 µL of Stop Solution to each well in the same order as addition of the TMB substrate. Mix by tapping the side of the plate gently. NB: Avoid skin and eye contact with the Stop solution.

8.10 Read results: Immediately after adding Stop solution read the absorbance on a microplate reader at a wavelength of 450 nm. If possible, perform a double wavelength readout (450 nm and 630 nm).

8.11 Data analysis: Calculate the average of the duplicate readings (OD value) for each standard and sample, and subtract the average of the zero standard absorbance. Construct a standard curve by plotting the mean absorbance for each standard on the y-axis against the concentration on the x-axis, use four-parameter logistic curve- fit (4-PL) analysis to do this. If the samples have been diluted, the OD readout from the standard curve must be multiplied by the dilution factor used.



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| Step | Reagent | Volume | Incubation | Wash | Notes |
|---|---|---|---|---|---|
| 1 | Additional diluent (Only for serum and plasma sample test) | 50 µL | 0 min | Do not wash | Add additional diluent 50 µL per well then add standard and samples immediately |
| 2 | Standard and Samples | 100 µL | 120 min | 4 times | Cover Wells incubate at 37°C |
| 3 | Diluent Detection antibody, HRP-conjugated Solution | 100 µL | 40 min | 4 times | Cover Wells incubate at 37°C |
| 4 | TMB Substrate | 100 µL | 15-20 min | Do not wash | Incubate in the dark at 37°C |
| 5 | Stop Solution | 100 µL | 0 min | Do not wash | - |
| 6 | Read plate at 450 nm and 630 nm immediately after adding Stop solution. DO NOT exceed 5 minutes. | | | | |



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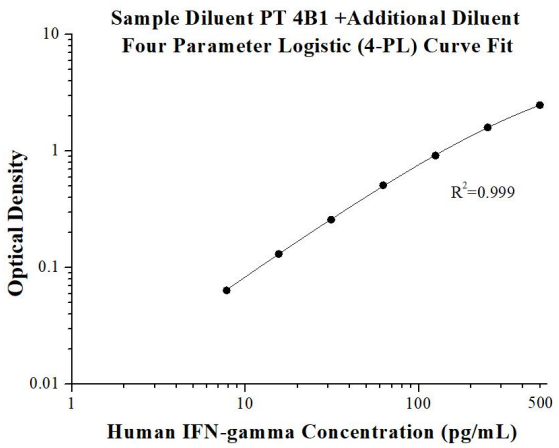
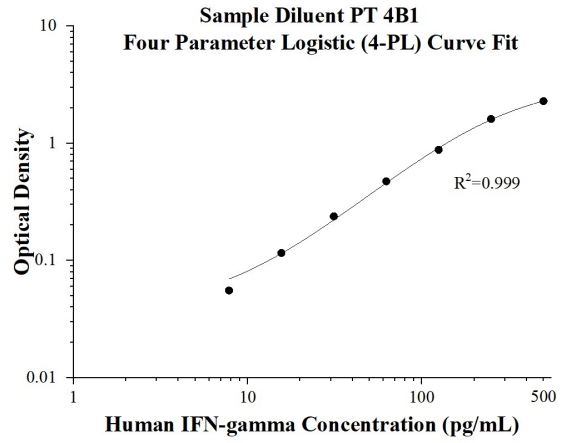
# 9. Validation Data

## 9.1 Standard curve

These standard curves are provided for demonstration only. A standard curve should be generated for each set of samples assayed.




| (pg/mL) | O.D | Average | Corrected |
|---|---|---|---|
| 0 | 0.0203<br>0.0202 | 0.02025 | - |
| 7.8 | 0.083<br>0.0851 | 0.08405 | 0.0638 |
| 15.6 | 0.1519<br>0.1508 | 0.1514 | 0.1311 |
| 31.25 | 0.2792<br>0.2775 | 0.2784 | 0.2581 |
| 62.5 | 0.533<br>0.5261 | 0.5296 | 0.5093 |
| 125 | 0.9645<br>0.9089 | 0.9367 | 0.91645 |
| 250 | 1.6215<br>1.6113 | 1.6164 | 1.5962 |
| 500 | 2.493<br>2.5064 | 2.4997 | 2.47945 |




| (pg/mL) | O.D | Average | Corrected |
|---|---|---|---|
| 0 | 0.0165<br>0.016 | 0.01625 | - |
| 7.8 | 0.0765<br>0.0668 | 0.07165 | 0.0554 |
| 15.6 | 0.1419<br>0.1223 | 0.1321 | 0.11585 |
| 31.25 | 0.2674<br>0.2404 | 0.2539 | 0.23765 |
| 62.5 | 0.502<br>0.4772 | 0.4896 | 0.47335 |
| 125 | 0.8942<br>0.8974 | 0.8958 | 0.87955 |
| 250 | 1.6114<br>1.6425 | 1.62695 | 1.6107 |
| 500 | 2.3064<br>2.304 | 2.3052 | 2.28895 |



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## 9.2 Precision

**Intra-assay Precision** (Precision within an assay) Three samples of known concentration were tested 8 times on one plate to assess intra-assay precision.

**Inter-assay Precision** (Precision between assays) Three samples of known concentration were tested in 16 separate assays to assess inter-assay precision.

| Intra-assay Precision | | | | |
|---|---|---|---|---|
| Sample | n | Mean (pg/mL) | SD | CV% |
| 1 | 8 | 242.2 | 10.6 | 4.4 |
| 2 | 8 | 61.5 | 3.1 | 5.1 |
| 3 | 8 | 33.5 | 1.3 | 4.0 |

| Inter-assay Precision | | | | |
|---|---|---|---|---|
| Sample | n | Mean (pg/mL) | SD | CV% |
| 1 | 16 | 246.4 | 16.3 | 6.6 |
| 2 | 16 | 63.9 | 3.9 | 6.0 |
| 3 | 16 | 33.8 | 1.5 | 4.3 |

## 9.3 Recovery

The recovery of human IFN-gamma spiked to three different levels throughout the range of the assay in various matrices was evaluated.

| Sample Type | | Average% of Expected | Range (%) |
|---|---|---|---|
| Human serum | 1:2 | 98 | 91-110 |
| Cell culture supernatant | 1:8 | 105 | 102-109 |
| | 1:16 | 98 | 93-103 |



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## 9.4 Sample values

**Human serum** - human serum samples were evaluated for the presence of human IFN-gamma in this assay.

| Sample Type | Mean (pg/mL) | Range (pg/mL) |
|---|---|---|
| Human serum (n=16) | 11.7 | 8.3-16.6 |

**Cell culture supernatant:**

Human peripheral blood mononuclear cells (PBMC) (1 x $10^6$ cells/mL) were cultured in DMEM supplemented with 10% fetal bovine serum, 5μM β-mercaptoethanol, 2 mM L-glutamine, 100 U/mL penicillin, and 100 μg/mL streptomycin sulfate. Cells were cultured stimulated with 10 ng/mL PMA and 500 ng/mL $Ca^{2+}$ for 1 day. Aliquots of the cell culture supernates were removed, assayed for human IFN-gamma, and measured 663.8 pg/mL.

Human peripheral blood mononuclear cells (PBMC) (1 x $10^6$ cells/mL) were cultured in DMEM supplemented with 10% fetal bovine serum, 5μM β-mercaptoethanol, 2 mM L-glutamine, 100 U/mL penicillin, and 100 μg/mL streptomycin sulfate. Cells were cultured unstimulated or stimulated with 10 μg/mL PHA for 1 day and 5 days. Aliquots of the cell culture supernates were removed and assayed for levels of human IFN-gamma.

| Condition | Day 1 (**ng/mL**) | Day 5 (**ng/mL**) |
|---|---|---|
| Unstimulated | ND | 0.3 |
| Stimulated | 21.0 | 79.6 |

ND*=Non-detectable

## 9.5 Sensitivity

The minimum detectable dose of human IFN-gamma is 1.1 pg/mL. This was determined by adding two standard deviations to the concentration corresponding to the mean O.D. of 20 zero standard replicates.



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## 9.6 Linearity

To assess the linearity of the assay, Human serum samples were spiked with high concentrations of human IFN-gamma and diluted with the appropriate **Sample Diluent** to produce samples with values within the dynamic range of the assay. Cell culture supernatant samples were diluted with the appropriate **Sample Diluent** to produce samples with values within the dynamic range of the assay.

| | | Human serum | Cell culture supernatant |
|---|---|---|---|
| 1:2 | Average% of Expected | 89 | 100 |
| | Range (%) | 88-90 | - |
| 1:4 | Average% of Expected | 91 | 100 |
| | Range (%) | 88-94 | 99-101 |
| 1:8 | Average% of Expected | 98 | 100 |
| | Range (%) | 95-100 | 98-102 |
| 1:16 | Average% of Expected | 97 | 99 |
| | Range (%) | 92-101 | 97-101 |

## 9.7 Calibration

The NIBSC/WHO Human IFN-gamma Reference Reagent (82/587) (rDNA derived), which was intended as a potency standard, was evaluated in this kit. The dose response curve of this Reference Reagent parallels the Proteintech standard curve. To convert sample values obtained with the Authentikine Human IFN-gamma ELISA kit to approximate NIBSC/WHO (82/587) values, use the equation below.

NIBSC/WHO (82/587) approximate value (IU/mL) = 0.02 x Quantikine HS IFN-gamma value (pg/mL)

## 9.8 Specificity

This assay recognizes natural and recombinant human IFN-gamma.

The following factors prepared at 50 ng/mL were assayed and exhibited no cross-reactivity or interference.

| Recombinant human: | Recombinant mouse: | Recombinant rat: |
|---|---|---|
| IFN-γ R1 | IFN-γ | IFN-γ |
| IFN-β | | |

## 10. References


1. Gray, P W, and D V Goeddel. Nature vol. 298,5877 (1982): 859-63.
2. Schoenborn, Jamie R, and Christopher B Wilson. Advances in immunology vol. 96 (2007): 41-101.
3. Denkinger, Claudia M et al. PloS one vol. 8,12 (2013): e85447.
4. Schroder, Kate et al. Journal of leukocyte biology vol. 75,2 (2004): 163-89.





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