Version: 1.01

Human Interferon gamma(IFN-γ) ELISA kit
Cat No: SB-EKH1097

Pack Size: 96Tests

FOR RESEARCH USE ONLY

NOT FOR INVITRO CLINICAL DIAGNOSTICS

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Intended Use

The kit is intended for use in quantitative determination of Human IFN-γ concentrations in serum, plasma, tissue homogenates, cell lysates, cell culture supernates and other biological fluids.

Specification

1. Sensitivity: 9.38 pg/mL.
2. Detection Range: 15.6-1000 pg/mL.
3. No significant cross-reactivity or interference between Human IFN-γ and analogues was observed.
4. Repeatability: Coefficient of variation is < 8%.

Principle of the Procedure

This kit uses the Sandwich-ELISA principle. The microtiter plate strips has been pre-coated with an affinity purified antibody to Human IFN-γ. Standards or samples containing Human IFN-γ are added to the plate and reacted with capture antibody. A second anti-Human IFN-γ antibody labeled biotin is then added and binds to Human IFN-γ captured on the plate. After that, Streptavidin-Horseradish Peroxidase(SA-HRP) is added to form a sandwich complex of solid phase antibody-Human IFN-γ-biotin labeled antibody-SA-HRP. And then, TMB substrate solution is added to all wells and incubated. An enzyme-catalyzed reaction generates a blue color in the solution, thereafter, stop solution is added to stop the substrate reaction and the color turns yellow. The yellow solution is read at a wavelength of 450nm. The concentration of Human IFN-γ in the samples is then calculated from the OD value by establishing a standard curve.

Reagents & Materials Provided

Components	Specification	Storage
Pre-coated Microtiter Plate	Ready to use, 8 wells ×12 strips	-20°C
Standard	Lyophilized, 2vials	-20°C
Detection Reagent A(100×)	Biotinylated Antibody, 1×120µL	-20°C
Detection Reagent B(100×)	SA-HRP enzyme conjugate, 1×120µL	-20°C
Standard & Sample Diluent	Dilution of samples and standards, 1× 20mL	2-8°C
Assay Diluent A	Dilution of Detection Reagent A, 1× 12mL	2-8°C
Assay Diluent B	Dilution of Detection Reagent B, 1× 12mL	2-8°C
Wash Buffer (30×)	Washing away unbound substances, 1× 20mL	2-8°C
TMB Reagent	Tetramethylbenzidine solution, 1× 9mL	2-8°C
Stop Solution	1N sulfuric acid, 1× 6mL	2-8°C
Plate Sealers	Sealing the microtiter well, 2 pieces	
Product Manual	ELISA kit operating instructions, 1 copy	
Certificate of Analysis	Product quality test results, 1 copy	

Storage & Stability

1. After receiving the kit, an unopened kit can be stored at 2-8℃ for 1 month. If the kit is not used within 1 month, store each component separately at the temperature indicated in the table above once the kit is received.
2. When the kit is used, the remaining reagents should be stored according to the table above storage condition.Unused plate strips should be immediately returned to the foil bags containing the desiccant, resealed and stored at -20℃.
3. All reagent bottle caps must be tightened to prevent evaporation and microbial pollution, the volume of imeasuring equipment instead of directly pouring into the vials.
4. The expiration date of the product is determined by the label on the box, and all components are guaranteed to be stable during the shelf life.

Materials & Equipment Required but Not Provided

1.Microplate reader with 450nm wavelength filter
2.Incubator capable of maintaining 37℃
3.Single or multi-channel pipettes with high precision
4.disposable pipette tips
5.EP tubes
6.Container for Wash Solution
7.Squirt bottle, manifold dispenser, or automated microplate washer
8.Deionized or distilled water
9.Absorbent paper for blotting the microplate

Sample Collection

- **Serum:** Allow blood samples to clot for 2 hours at room temperature or overnight at 2-8℃ before centrifugation for 15 min at 2000×g at 2-8℃. Supernatants should be taken for assay testing immediately or stored at -20℃ or -80℃ for later use.

- **Plasma :** Collect plasma using EDTA or heparin as an anticoagulant. Centrifuge samples for 15 min at 2000×g at 2-8℃ within 30 min of collection. Supernatants should be taken for assay testing immediately or stored at -20℃ or -80℃ for later use.

- **Tissue homogenates :** Tissues should be rinsed in cool PBS to remove excess blood thoroughly, weighed, minced into small pieces. then Tissue pieces homogenized in cool PBS (tissue weight (g): PBS (mL) volume=1:9) with a glass homogenizer on ice. The resulting suspension is solicited with an ultrasonic cell disrupter till the solution is clarified. The homogenates are then centrifuged for 5 min at 10000×g. Supernatants should be taken for assay testing immediately or stored at -20℃ or -80℃ for later use.

- **Cell lysates:** For adherent cells, gently wash the cells with moderate amount of cool PBS and detach the cells with trypsin. Collect cells by centrifugation for 5 min at 1500×g(suspension cells can be collected by centrifugation directly). Discard the supernate and wash cells 3 times with cool PBS. Resuspend cells in cool PBS with concentration of 5×106 cells/mL. Repeat the freeze-thaw process several times until the cells are fully lysed. Centrifuge for 15min at 2000×g at 2-8℃. Supernatants should be taken for assay testing immediately or stored at -20℃ or -80℃ for later use.

- **Cell culture supernatant and other biological fluids:** Centrifuge samples for 15 min at 1500×g at 2-8℃. Supernatants should be taken for assay testing immediately or stored at -20℃ or -80℃ for later use.

Reagent Preparation

1.Allow all reagents to return to room temperature (18-25℃) before use.

2.**Wash Buffer**: Dilute 20 mL of Concentrated Wash Buffer with 580 mL of deionized or distilled water to prepare 600 mL of Wash Buffer.

3.Standard Working Solution: First, centrifuge the standard at 1000×g for 1 min, add 1mL of standard sample dilution, let it stand for 10min and then mix gently, that is 1000pg/mL of standard working solution. Second, take 7 EP tubes, add 500μL of Standard & Sample Diluent to each tube. Pipette 500μL of the 1000pg/mL working solution to the first tube and mix up to produce a 500pg/mL working solution. Pipette 500μL of the solution from the former tube into the latter one according to the picture shown below. Mix each tube thoroughly before the next transfer. Set up 7 points of diluted standard such as 1000pg/mL, 500pg/mL, 250pg/mL, 125pg/mL, 62.5pg/mL, 31.2pg/mL, 15.6pg/mL, and the last EP tubes with Standard & Sample Diluent is the blank as 0pg/mL.

4.Detection Reagent A working solution: Calculate the required amount before the experiment (100 μL/well). In preparation, slightly more than calculated should be prepared. Centrifuge the stock tube before use, dilute the 100× Concentrated Detection Reagent A to 1×working solution with Assay Diluent A.

5. Detection Reagent B working solution: Calculate the required amount before the experiment (100 μL/well). In preparation, slightly more than calculated should be prepared. Centrifuge the stock tube before use, dilute the 100× Concentrated Detection Reagent B to 1× working solution with Assay Diluent B.

Assay Protocol

1. Add the Standard working solution to the first two columns: Each concentration of the solution is added in duplicate, to one well each, side by side (100 μL for each well). Add the samples to the other wells (100 μL for each well). Cover with the plate sealer provided in the kit. Incubate for 60 min at 37℃.

2. Discard the liquid from each well, don't wash. Immediately add 100μL of Detection Reagent A working solution to each well. Cover with the plate sealer. Incubate for 60 min at 37℃.

3. Discard the liquid from each well, add 350μL of wash buffer to each well. Soak for 30sec and decant the solution from each well and pat it dry against clean absorbent paper. Repeat this wash step 3 times in total.

4. Add 100μL of Detection Reagent B working solution to each well. Cover with the plate sealer. Incubate for 30 min at 37℃.

5. Turn on the Plate Reader for preheating.

6. Discard the liquid from each well, repeat the washing process of step 3 for 5 times.

7. Add 90μL of TMB Reagent to each well. Cover with a new plate sealer. Incubate for 10-20min at 37℃. Protect the plate from light.

8. Add 50 μL of Stop Solution to each well

9. The absorbance OD value of each well is measured at 450nm right away.

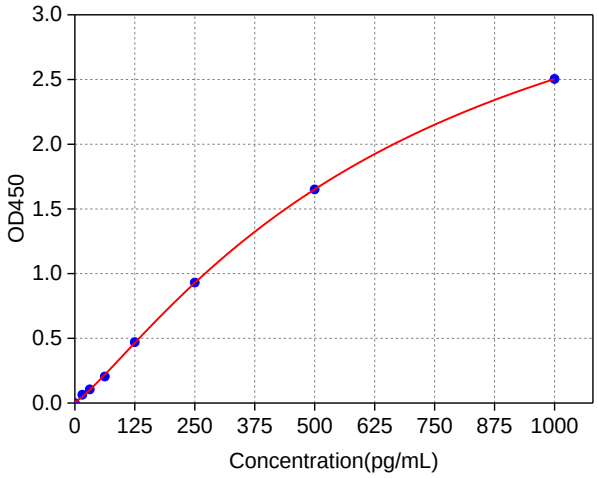
Example data

The OD values of the standard curve may vary according to the conditions of the actual assay performance (e.g. operator, pipetting technique, washing technique or temperature effects), so plotting log of the data to establish a standard curve for each test is strongly recommended. Typical standard curve is provided below for reference only

Concentration(pg/mL)		0	3.13	6.25	12.5	25	50	100	200
OD450nm	1	0.055	0.096	0.18	0.424	0.823	1.261	1.837	2.393
	2	0.047	0.1	0.196	0.414	0.779	1.217	1.8522	2.497
Mean OD		0.051	0.098	0.188	0.419	0.801	1.239	1.8446	2.445
Corrected OD		0	0.047	0.137	0.368	0.75	1.188	1.7936	2.394

Calculation of Results

Average the duplicate readings for each standard and samples, then subtract the average zero standard optical density. Create a standard curve with standard concentration on the x-axis and OD values on the y-axis. Draw a best fit curve through the points and it can be determined by regression analysis. If samples have been diluted, the concentration read from the standard curve must be multiplied by the dilution factor.



Sensitivity

The minimum detectable dose of Human IFN-γ is typically less than 9.38pg/mL.
The sensitivity of this assay, or Limit of Detection (LOD) was defined as the lowest protein concentration that could be differentiated from zero. It was determined by adding three standard deviations to the mean OD value of twenty zero standard replicates and calculating the corresponding concentration.

Specificity

This assay has high sensitivity and excellent specificity for detection of Human IFN-γ.
No significant cross-reactivity or interference between Human IFN-γ and analogues was observed.
Limited by current skills and knowledge, it is impossible for us to complete the cross- reactivity detection between Human IFN-γ and all the analogues, therefore, cross reaction may still exist.

Precision

Mean coefficient of variation for Intra-Assay and Inter-Assay: 3 samples with low, middle and high level concentration were tested for repeat multiple times, respectively.

Item	Intra-Assay	Inter-Assay
Sample number	3	3
Replicate	9	18
CV	4%	8%

Recovery

Three matrices listed below were spiked with certain level of Human IFN-γ, The recovery rates of Human IFN-γ were calculated by comparing the measured value to the expected amount of Human IFN-γ in samples.

Matrix type	**Recovery Range (%)**	**Average (%)**
Serum (n=5)	85-101	92
EDTA plasma (n=5)	86-96	91
Cell culture media (n=5)	94-110	101

Linearity

Three types of Sample were spiked with appropriate concentrations of Human IFN-γ and diluted into a series of concentration gradients, then the linearity of the assay was demonstrated by the percentage of comparing calculated concentrations and expected values.

Dilution Factor	**Recovery Range (%)**		
	Serum (n=5)	EDTA plasma (n=5)	Cell culture media (n=5)
1:2	84-97	90-104	97-114
1:4	81-93	92-108	95-109
1:8	83-94	92-104	98-111
1:16	81-92	88-102	98-114

Summary

Prepare all reagents

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Add 100μL **Standard or Sample**. Incubate 60mintues at 37℃

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Discard the liquid. Add 100μL **Detection Reagent A**
Incubate 60mintues at 37℃

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Aspirate and wash 3 times

↓

Add 100μL **Detection Reagent B**. Incubate 30 minutes at 37℃

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Aspirate and wash 5 times

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Add 90μL **TMB Reagent**. Incubate 15 minutes at 37℃

↓

Add 50μL Stop Solution

↓

Read at 450nm immediately and calculate of results