Mouse IFN-γ ELISA Kit

Product description

Mouse interferon-gamma (Mouse IFN-γ) is a soluble homodimeric cytokine and the sole member of the type II interferon family. It is mainly secreted by natural killer (NK) cells and natural killer T (NKT) cells, playing a crucial role in immune function. Interferon-gamma (IFN-γ) is essential for innate and adaptive immunity against viral, bacterial, and parasitic infections. It acts as a vital activator of macrophages and induces the expression of major histocompatibility complex class II (MHC II). Aberrant expression of IFN-γ is associated with many autoimmune and inflammatory diseases. Besides directly inhibiting viral replication, IFN-γ is important for immune stimulation and regulation. It can effectively inhibit cell proliferation and is used clinically to treat various conditions such as primary thrombocythemia, chronic myeloid leukemia, polycythemia vera, and primary myelofibrosis.

The Arcegen Mouse IFN-γ ELISA assay kit is an in vitro enzyme-linked immunosorbent assay (ELISA) kit used for quantitative measurement of mouse interferon-gamma (Mouse IFN-γ) in serum and plasma. Specific anti-mouse interferon-gamma antibodies are precoated on a high-affinity enzyme-linked immunosorbent assay (ELISA) plate. Standard samples and test samples are added to the wells of the ELISA plate, and after incubation, mouse interferon-gamma present in the samples binds to the solid-phase antibody. After washing to remove unbound substances, a detection antibody is added for incubation, followed by washing. Streptavidin-horseradish peroxidase (HRP) conjugate is then added for incubation. After another washing step, a colorimetric substrateTMB is added for color development. The intensity of the color reaction is proportional to the concentration of mouse interferon-gamma in the sample. The reaction is terminated by adding a stop solution, and the absorbance is measured at 450 nm wavelength (with a reference wavelength range of 570 - 630 nm).

Specifications

Item Number	P162007S / P162007E
Specification	48 T / 96 T
Detection Range	31.25-2000 pg/mL
Detection Method	Sandwich ELISA
Detection Time	4.5 hours
Sensitivity	5.65 pg/mL
Dilution Linearity	82 - 120%
Recovery Rate	79 - 108%
Intra-assay Variability	4.5%

Inter-assay Variability	6.4%

Components

Component Number	Component Name	Storage Temperature	P162007S	P162007E
P162007-A	ELISA Plate	2~8°C	48 T	96 T
P162007-B	Standard	2~8°C	1 tube	2 tubes
P162007-C	Detection Antibody	2~8°C	120 µL	240 µL
P162007-D	Enzyme Conjugate	2~8°C(Avoid Light)	30 µL	60 µL
P162007-E	Sample Dilution Buffer	2~8°C	8 mL	15 mL
P162007-F	Antibody/Enzyme Dilution Buffer	2~8°C	15 mL	30 mL
P162007-G	20x Wash Buffer	2~8°C	25 mL	50 mL
P162007-H	Substrate Solution	2~8°C(Avoid Light)	8 mL	15 mL
P162007-I	Stop Solution	Room Temperature	5 mL	10 mL
P162007-J	Plate Sealant Film	Room Temperature	3 pieces	5 pieces

Storage

The kit can be stored at 2~8°C or according to the storage conditions provided for each component to avoid contamination and repeated freeze-thaw cycles. Diluted working concentration reagents should be used immediately and discarded after use; they should not be reused. The shelf life is 1 year.

Table 1. Reagent Storage Table After Initial Use

Component Name	Storage Conditions
ELISA Plate	Unused strips can be returned to the aluminum foil bag,tightly sealed,and stored at 2-8°C to avoid moisture absorption.
Standard	Use within 48 hours after dissolution,store at 2-8°C to avoid contamination.
Detection Antibody	Use within 48 hours after dilution,store at 2~8°C to avoid contamination.
Enzyme Conjugate	
Sample Dilution Buffer	Store at 2~8°C for 1 month,avoiding contamination.
Antibody/Enzyme Dilution Buffer	
20x Wash Buffer	
Substrate Solution	Store at 2~8°C for 1 month,avoiding light exposure.
Stop Solution	Can be stored at room temperature.

Plate Sealant Film	

Instructions

1. Used for quantitatively detecting the IFN-γ content in serum, plasma, and cell culture supernatant.
2. Please read the instruction manual carefully before using this product.

Notes

1. This product is for research use only.
2. For your safety and health, please wear laboratory coats and disposable gloves when handling.
3. The kit should be used within its shelf life. Mixing related reagents from different batches is prohibited.
4. This product is intended for detecting the target antigens and samples as indicated in the instruction manual. Other applications should be validated and evaluated for reliability and accuracy by the user based on the results.
5. Do not mix or substitute reagents or materials from different batches of other kits.

Common Technical Tips

1. If the sample OD value is higher than the S1 OD value, further dilute it in an appropriate sample dilution buffer.
2. Avoid generating foam during mixing.
3. When adding standard samples, specimens, and others, change the tips promptly to avoid cross-contamination.
4. Ensure proper sealing of the microplate or complete coverage with plate sealing film during the incubation period.
5. Completely remove all solutions and buffers during the washing steps.
6. Do not invert the standard sample tube before dissolving the standard. After inverting the standard sample tube, thoroughly mix it up and down after adding the buffer, then centrifuge at low speed.
7. During the experiment, store the reagents as instructed in the manual.
8. Dispose of the buffer promptly after completing the experiment; use it once and discard.
9. Different product kits have different components and should not be cross-used.

Other Preparation Materials

1. ELISA reader, measure absorbance at 450 nm (reference wavelength 630 nm).
2. Incubator, automated microplate washer.

3. Pipettes, 1 μL to 1 mL pipettes with corresponding tips.
4. 100 mL and 1 L graduated cylinders.
5. Standard or sample dilution tubes.
6. Blotting paper.
7. Distilled water or deionized water.
8. Computer and analysis software.

Pre-Experiment Preparation

1. Sample Collection and Processing

1) Cell culture supernatant: Centrifuge at 1, 000×g for 10 minutes to remove precipitates. Immediate testing or aliquoting, and store at -20°C or below.

2) Serum samples: Collect serum using tubes without pyrogens and endotoxins. Let the blood clot for 30 minutes, then centrifuge at 1, 000×g for 10 minutes. Test immediately after aspirating the serum sample or aliquot and store at -20°C or below.

3) Plasma samples: Collect plasma samples using EDTA, sodium citrate, or heparin anticoagulants. Centrifuge at 1, 000 × g for 30 minutes to collect samples. Test immediately or aliquot and store at -20°C or below.

This assay kit may be applicable to other biological samples. Serum, plasma, and cell culture supernatants have been validated.

[Note]Before testing, visible precipitates in the samples must be removed. Do not use severely hemolyzed or lipemic samples. Samples should be aliquoted and stored at -20°C to avoid the loss of IFN-γ activity. If testing within 24 hours, samples can be stored at 2~8°C to avoid repeated freeze-thaw cycles. Before testing, frozen samples should be slowly equilibrated to room temperature (25°C ± 3°C) and gently mixed.

If samples need to be diluted, use the specified sample dilution buffer for dilution.

Recommended dilution for normal serum/plasma samples (for reference only): Use sample dilution buffer at a 1:1 dilution. Recommended for cell culture supernatants (for reference only): Undiluted.

Due to variations in the target protein content in samples, the dilution ratio for each sample is recommended based on pre-experimental results or determined based on actual conditions.

2. Preparation of the ELISA Plate

The ELISA plate should be brought to room temperature before use.Unused strips should be promptly sealed in a desiccant and stored at 2~8°C, with each sample requiring multiple wells for experimentation.

3. Reagent Preparation

Before use, all reagents and samples need to be equilibrated to room temperature. To ensure the

accuracy of the experiment, please complete the equilibration within 15 minutes before use.

1) 1× Wash Buffer Preparation: Balance the concentrated solution to room temperature and dissolve completely without crystallization. Mix well, take 25 mL of 20× Wash Buffer and dilute with distilled water, then bring to a final volume of 500 mL; Specific preparation volumes can be adjusted according to the amount needed for each use.

2) Detection Antibody Preparation: Before use, centrifuge at 10, 000 rpm for 20 seconds, then dilute with Antibody Dilution Buffer at a 1:50 ratio to the working concentration. For example, take 120 µL and bring to a final volume of 6 mL with Antibody Dilution Buffer; Specific preparation volumes can be adjusted according to the amount needed for each use, and mix well.

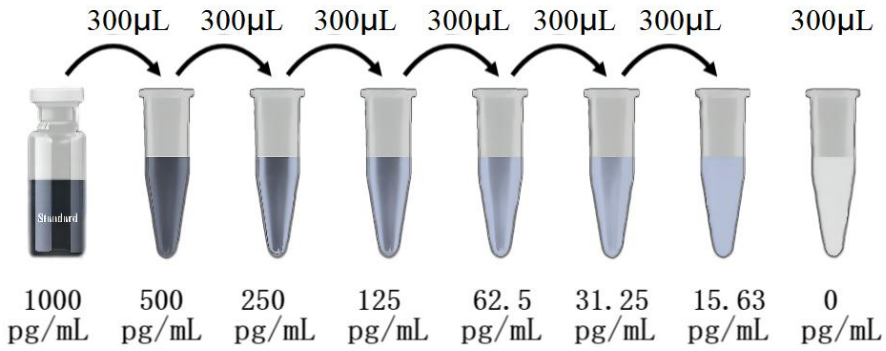
3) Enzyme Conjugate Preparation: Before use, centrifuge at 10, 000 rpm for 20 seconds, then dilute with Enzyme Dilution Buffer at a 1:200 ratio to the working concentration. For example, take 30 µL and bring to a final volume of 6 mL with Antibody/Enzyme Dilution Buffer; Specific preparation volumes can be adjusted according to the amount needed for each use, and mix well.

Preparation of standard curve: Prepare 7 sterile 1.5mL centrifuge tubes and label them according to the standard concentrations. For the preparation of S1: dissolve an appropriate amount of standard lyophilized substance in sample diluent as indicated on the label, mix thoroughly, and label it as 2000 pg/mL. Add 300µL of 1× sample diluent to each centrifuge tube. Take 300µL of S1 and add it to the first centrifuge tube, mix thoroughly, then take 300µL and transfer it to the next tube labeled with the next concentration, mix thoroughly. Perform a series of 2-fold gradient dilutions of the standard substance, starting with the highest concentration labeled as 2000 pg/mL and ending with the lowest concentration of 31.25 pg/mL, following the preparation method below. Prepare a corresponding standard curve for each assay, and do not mix standard curves from different reagent kits or different time points. For sample testing, add 100µL of standard substance to each well. Ensure that the volume prepared is higher than the required volume to avoid insufficient usage.

Table 2. IFN-γ Standard Curve Preparation (31.25 - 2000 pg/mL)

Standard Curve	Diluent (µL)	Volume of Standard Added (µL)	Final Concentration of Standard (pg/mL)
S1	As labeled	/	1000
S2	300	300	500
S3	300	300	250
S4	300	300	125
S5	300	300	62.5
S6	300	300	31.25
S7	300	300	15.63

Blank	300	0	0



Operating Instructions

Before use, all reagents and samples need to be equilibrated to room temperature. It is strongly recommended to perform duplicate well testing for all standards and test samples.

1. Reagent Preparation: Prepare various test reagents, dilute standard solutions, and prepare test samples.
2. Plate Determination: Calculate the number of plates needed for test samples and standards. Remove the plate from the aluminum foil bag, return the remaining plates to the bag, seal the bag, and store at low temperature.
3. Plate Soaking: Add 350 μL of 1× washing solution per well to soak the plate. After standing for 30 seconds, remove the liquid from the wells and tap-dry the plate. The volume of liquid is crucial for the test results, so ensure that there is no residual washing solution after the final tapping.
4. Sample Incubation: Add various gradient standard solutions and diluted test samples to the plate, 100 μL per well. Ensure that the spotting is completed within 15 minutes and incubate at room temperature for 2 hours.
5. Plate Washing: Discard the liquid from the wells, wash the plate 5 times with 350 μL of 1× washing solution per well, and tap-dry the plate.
6. Antibody Incubation: Add the detection antibody pre-diluted to the working concentration to the plate, 100 μL per well, and incubate at room temperature for 2 hours.
7. Plate Washing: Discard the liquid from the wells, wash the plate 5 times with 350 μL of 1× washing solution per well, and tap-dry the plate.
8. Enzyme Conjugate Incubation: Add the enzyme conjugate pre-diluted to the working concentration to the plate, 100 μL per well, and incubate at room temperature for 20 minutes.
9. Plate Washing: Discard the liquid from the wells, wash the plate 5 times with 350 μL of 1× washing solution per well, and tap-dry the plate.
10. Color Development: Allow the substrate solution to equilibrate to room temperature for 10 minutes before use. Add 100 μL of substrate solution to each well of the plate and incubate at room temperature in the dark for 15 minutes.

11. Termination: Add 50 µL of stop solution to each well of the plate. The color will change from blue to yellow. Gently shake the plate to ensure uniform color development.
12. Reading: Read the absorbance values at 450 nm/630 nm within 10 minutes.

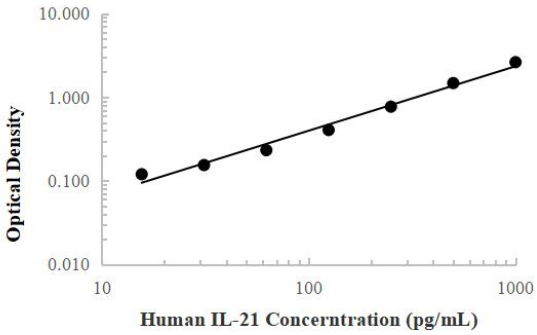
Standard Curve Establishment

Calculate the average OD values of duplicate wells for both standards and samples, and subtract the average OD value of blank wells to obtain the calibrated OD value. Plot the standard curve with the logarithm of standard concentrations on the x-axis and the logarithm of calibrated OD values on the y-axis. Various plotting and statistical software can be used to assist in plotting the standard curve and calculating the concentration of unknown samples. The four-parameter fitting method often yields better fitting results, while other methods such as linear fitting may also yield good fitting results, depending on the specific experimental data for analysis.

Experimental Data

1. Standard Curve Data

Fitting data to generate a standard curve for analysis of experimental data.



Standard Curve Graph

Concentration(pg/mL)	Absorbance		Mean Value	Calibration Value
2000	2.560	2.579	2.569	2.572
1000	1.892	1.879	1.886	1.878
500	1.271	1.193	1.232	1.240
250	0.780	0.726	0.753	0.755
125	0.472	0.410	0.441	0.437
62.5	0.263	0.241	0.252	0.248
31.25	0.142	0.137	0.139	0.141

2. Sensitivity Detection

The minimum detection limit of IFN-γ is 5.65 pg/mL, calculated using the mean and standard deviation of the OD values from 20 repeated measurements of the blank wells.

3. Precision Detection

Intra-assay Precision

Three samples of known concentrations were assayed 8 times each to evaluate the precision within the ELISA plate.

Inter-assay Precision

Three samples of known concentrations were assayed 24 times each on different ELISA plates to evaluate the precision between ELISA plates.

Project	Intra-assay Precision			Inter-assay Precision		
Sample	1	2	3	1	2	3
	8	8	8	24	24	24
Mean Value	1093.5	274.5	61.2	1062.9	272.2	59.8
Standard Deviation	70.3	8.5	2.4	100.6	15.9	2.3
Coefficient of Variation (%)	6.4	3.1	3.9	9.5	5.9	3.9

4. Recovery Rate Detection

The recovery rate was determined by adding different levels of IFN-γ to the samples, and the results are as follows:

Sample Types	Average Recovery Rate (%)	Range (%)
Serum	84.3	80.3-89.1
Plasma	87.5	79.1-97.5
Cell culture supernatant	98.7	86.4-108.6

5. Dilution Linearity Test

Serum Dilution Ratio	Mean Expected Value (%)	Range (%)
1:02	100.9	91.7-112.3
1:04	100.9	94.3-107.0
1:08	108.6	96.7-119.3
1:16	117.8	115.9-120.2

Plasma Dilution Ratio	Mean Expected Value (%)	Range (%)
1:02	106.8	96.3-113.0
1:04	97.6	93.0-103.1
1:08	108.1	104.6-114.4
1:16	112.8	111.4-115.6

Cell Culture Supernatant Dilution Ratio	Mean Expected Value (%)	Range (%)
1:02	91.1	87.0-94.1
1:04	96.5	93.9-101.3
1:08	99.7	92.1-104.3
1:16	90.3	82.4-104.1

6. Sample Values

Using this assay kit, samples from several healthy volunteers were tested, with no detailed medication history available for the volunteers.

Sample Types	Sample Number	Mean Value (mg/mL)	Sample Value(mg/mL)
Serum	4	n.d.	n.d.
Plasma	4	51.1	n.d.-118.6
Cell Culture Supernatant	4	28.3	n.d.-66.8

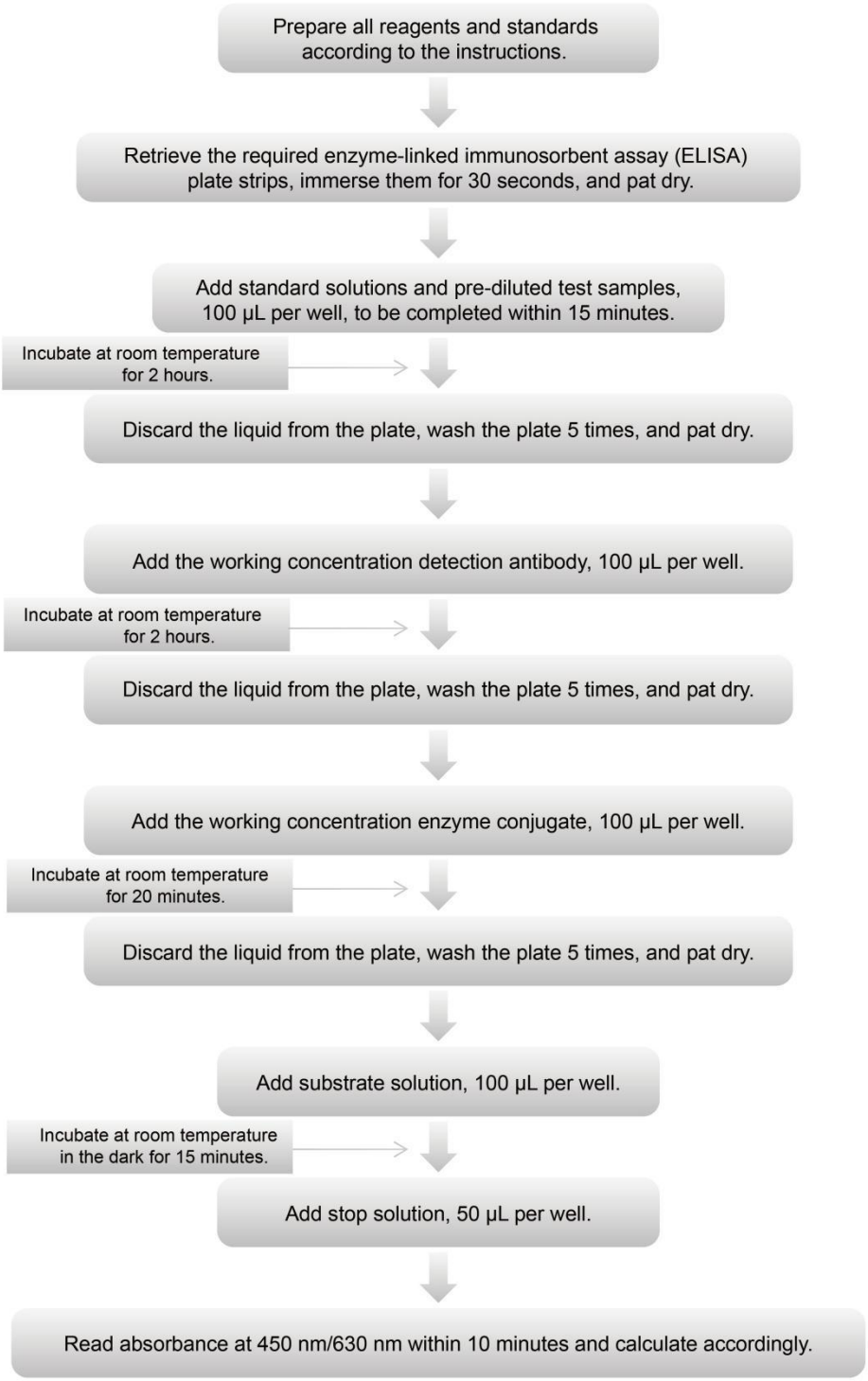
n.d. refers to sample concentrations below the detection range of 31.25 pg/mL.

7. Specificity Assay

This assay kit recognizes both natural and recombinant mouse interferon-gamma (IFN-γ). Specific evaluations of the following factors were conducted, and no significant cross-reactivity or interference effects were observed.

Recombinant human:		Recombinant Mouse:		Recombinant human:	
IFN-γ	IL-6	IL-1α	IL-10	IFN-γ	IL-6
IL-1α	IL-10	IL-1β	IL-12	IL-1α	IL-10
IL-1β	IL-12	IL-2	IL-13	IL-1β	IL-12
IL-2	IL-13	IL-3	TNF-α	IL-2	IL-13
IL-3	TNF-α	IL-4	VEGF	IL-3	TNF-α
IL-4	VEGF	IL-6		IL-4	VEGF

Detection Schematic



Frequently Asked Questions

Issues	Causes	Solution
Poor standard curve	Inaccurate pipetting volumes	Check the pipette, calibrate it regularly, handle it carefully, tightly cap the tip while mixing thoroughly, and minimize foam formation.
	Inappropriate dilution methods	
Low color intensity	Insufficient incubation time	Allow sufficient incubation time, and replace samples and reconstituted standards overnight.
	Inadequate pipetting volumes or improper dilution	Calibrate pipettes and standardize operations.
High coefficient of variation (CV)	Improper washing of the ELISA plate	Use the correct washing procedure; if using a plate washer, check all ports for blockages.
	Contaminated washing solution	Prepare fresh washing solution.
Low sensitivity	Improper storage of the assay kit	Store according to the product component table.