ClinMax™ Human IFN-gamma Quick ELISA Kit

Catalog Number：CEA-C245

Assay Tests: 96 tests

IMPORTANT: Please carefully read this user guide before performing your experiment.

Product information

This kit is specifically designed for the accurate quantitation of human IFN-gamma from cell culture supernates, serum and plasma.

The principle of this assay employs a quantitative sandwich enzyme immunoassay approach. Initially, a microplate is coated with a capture antibody. Then, samples and biotinylated capture antibody are added to the wells. After the removal of any unbound materials through washing, streptavidin-HRP (SA-HRP) conjugate is added to the wells. Streptavidin has a very high affinity for biotin, so it binds to the biotinylated capture antibody that is already bound to the target antigen. After washing, a substrate specific to HRP is added to the wells. HRP catalyzes a reaction that converts the substrate into a detectable signal, often a color change or luminescence, depending on the substrate used. This enzymatic reaction amplifies the signal, allowing for higher sensitivity in detecting the target analyte. The intensity of the signal is measured using a spectrophotometer.

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NOTE:

1. This kit is for research use only and is not for use in diagnostic or therapeutic applications.
2. Please do not use the kit after the expiration date indicated on the kit label.
3. Do not mix or substitute reagents with those from other lots or sources.

Manufactured and distributed by
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Contents

The kit contains sufficient reagents for 96 wells.

Catalog	Contents	Amount
CEA245-C01	Pre-coated Anti-IFN-γ Antibody Microplate	1 plate
CEA245-C02	Human IFN-γ Standard	35 ng×2
CEA245-C03	Biotin-Anti-IFN-γ Antibody	25 μg
CEA245-C04	Biotin-Antibody Dilution Buffer	8 mL
CEA245-C05	Streptavidin-HRP Con. Solution	500 μL
CEA245-C06	Streptavidin-HRP Dilution Buffer	15 mL
CEA245-C07	20× Washing Buffer	50 mL
CEA245-C08	Sample Dilution Buffer	15 mL×2
CEA245-C09	Substrate Solution	12 mL
CEA245-C10	Stop Solution	6 mL

Storage

Keep the unopened kit stored at 2-8 °C. Avoid using the kit beyond its expiration date.
For opened kit and reconstituted reagents, with the exception of the two contents listed in following table, others can be stored for up to 30 days at 2-8 °C.

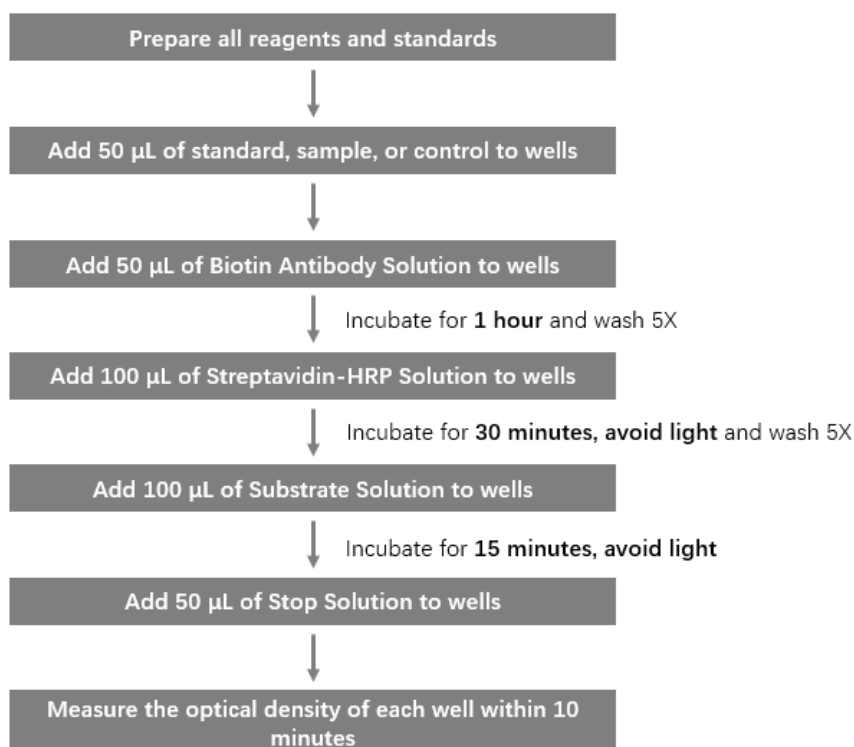| Contents	Storage conditions
Pre-coated Anti-IFN-γ Antibody Microplate	Return unused wells to the foil pouch, reseal along entire edge. May be stored for up to 1 month at 2-8°C.
Human IFN-γ Standard	Aliquot and store for up to 1 month at -70°C in a freezer. Avoid repeated freeze-thaw cycles.
Biotin-Anti-IFN-γ Antibody	Aliquot and store for up to 1 month at -70°C in a freezer. Avoid repeated freeze-thaw cycles.

NOTE: Streptavidin-HRP Con. Solution and Substrate Solution should avoid light.

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Required materials not supplied.

Instrument	Microplate reader capable of measuring absorbance at 450 nm
Reagents	Deionized, ultrapure or distilled water
Consumables	50 mL and 500 mL graduated cylinders
	Pipettes and pipette tips
	Tubes to prepare standard dilutions.

Workflow

Analyte:IFN-γ

Prepare all reagents and standards

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Add 50 μL of standard, sample, or control to wells

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Add 50 μL of Biotin Antibody Solution to wells

↓ Incubate for **1 hour** and wash 5X

Add 100 μL of Streptavidin-HRP Solution to wells

↓ Incubate for **30 minutes, avoid light** and wash 5X

Add 100 μL of Substrate Solution to wells

↓ Incubate for **15 minutes, avoid light**

Add 50 μL of Stop Solution to wells

↓

Measure the optical density of each well within 10 minutes

NOTE: Incubation temperature is 18 ℃-25 ℃

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Prepare the working buffers and standard dilutions.

IMPORTANT: Bring all reagents to room temperature before use. If crystals have formed in buffer solution, place the buffer solution in an 37°C incubator until the crystals have completely dissolved and bring the solution back to room temperature before use.

Prepare the reconstruct antibody stock solution.

Add 125 μL ultrapure water to the provided lyophilized product（CEA245-C03）, dissolve at room temperature for 15-30 minutes, and mix by gently pipetting. The concentration of reconstituted IFN-γ antibody stock solution is 200 μg/mL.

NOTE: Avoiding vigorous shaking or vortexing. The reconstituted solution should be stored at -70°C. The freeze-thaw cycle should not exceed 1 time, and the size of the aliquot should not be less than 10 μg.

Prepare the working buffers.

1. 1×Washing Buffer: Dilute 50 mL 20×Washing Buffer with deionized or distilled water to 1000 mL.

2. Biotin Antibody Solution: Add 30 μL of the reconstituted IFN-γ antibody stock solution to 6 mL Biotin-Antibody Dilution Buffer, thoroughly mix. The solution was freshly prepared just before use.

3. Streptavidin-HRP Solution: Add 480 μL of Streptavidin-HRP Con. Solution to 12 mL of Streptavidin-HRP Dilution Buffer, thoroughly mix. The solution was freshly prepared just before use.

Prepare the reconstituted standard.

Add 1 mL ultrapure water to the provided lyophilized product（CEA245-C02）, dissolve at room temperature for 15-30 minutes, and mix by gently pipetting. The concentration of reconstituted human IFN-γ Standard is 35 ng/mL.

NOTE: Avoiding vigorous shaking or vortexing. The reconstituted solution should be stored at -70°C. The freeze-thaw cycle should not exceed 1 time.

Prepare the standard serial dilutions.

1. Label a tube "Std.-1". Add 100 μL of the reconstituted human IFN-γ Standard and 900 μL of Sample Dilution Buffer to tube Std.-1, gently mix well (Std.-1 =3500 pg/mL).

2. Label 6 tubes, one for each standard point: Std.-2, Std.-3, Std.-4, Std.-5, Std.-6, Std.-7.

3. Prepare serial dilutions for the standard curve as follows: Add 500 μL of Sample Dilution Buffer to each tube (Std.-2, Std.-3, Std.-4, Std.-5, Std.-6, Std.-7).

4. Transfer 500 μL of liquid from Std.-1 to the tube Std.-2, and thoroughly mix (Std.-2 = 1750 pg/mL).

5. Continue to transfer 500 μL of liquid from previous dilution tube to the next dilution tube until add liquid to tube Std.-7.

6. Sample Dilution Buffer serves as zero standard (blank).

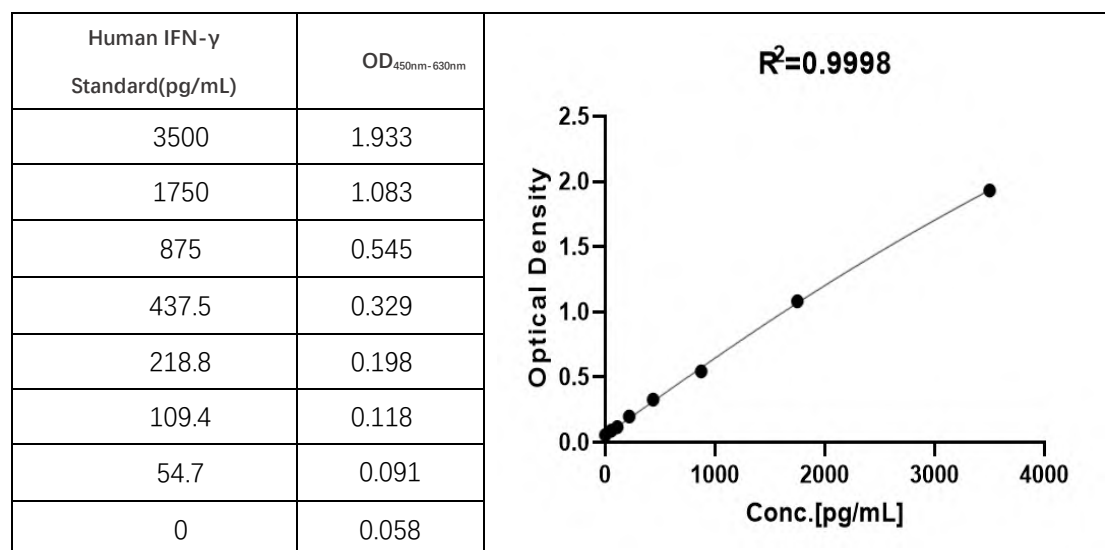*Human IFN-gamma Quick ELISA Kit User Guide*

PROCEDURE OF ASSAY

1. Add 50 µL of IFN-γ Standard, sample, or control to wells.

2. Add 50 µL Biotin Antibody Solution to each well, Seal the plate with microplate sealing film. Incubate at room temperature (18-25 °C) for **1 hours.**

3. Aspirate each well and add 300 µL of 1×Washing Buffer to each well, gently tap the plate for **1 minute**. Remove any remaining Washing Buffer by aspirating or decanting. Invert the plate and blot it against clean paper towels. Repeat the wash process four times for a total of five washes.

4. Add 100 µL of Streptavidin-HRP Solution to each well. Seal the plate with microplate sealing film. Incubate at room temperature (18-25 °C) for **30 minutes, avoid light.**

5. Repeat step 3.

6. Add 100 µL of Substrate Solution to each well. Seal the plate with microplate sealing film and incubate at room temperature (18-25 °C) for **15 minutes, avoid light**.

7. Add 50 µL of Stop Solution to each well. Tap the plate gently to ensure thorough mixing.

   Note: *the color in the wells should change from blue to yellow.*

8. Read the absorbance at 450nm and 630nm using Microplate reader within 10minutes.

   Note: *To reduce the background noise, subtract the readings at 630nm from the readings at 450nm.*

CALCULATION OF RESULTS

1. Compute the average of the duplicated readings for every standard, control, and sample. Then, subtract the average optical density (O.D.) of the zero standard(blank).
2. Establish a standard curve by processing the data using computer software capable of executing a four-parameter logistic (4-PL) curve fitting.
3. Normal range of Standard curve: $R^2 \geqslant 0.9900$.
4. If the OD value of the sample to be tested is higher than the highest standard, the sample shall be diluted with dilution buffer and assay repeated.

Typical data

Note: *For each experiment, a standard curve needs to be set for each microplate, and the specific OD value may vary depending on different laboratories, testers, or equipment. The following example data is for reference only. The sample concentration was calculated based on the results of the standard curve.*

Human IFN-γ Standard(pg/mL)	$OD_{450nm-630nm}$
3500	1.933
1750	1.083
875	0.545
437.5	0.329
218.8	0.198
109.4	0.118
54.7	0.091
0	0.058

PERFORMANCE CHARACTERISTICS

1. Sensitivity

The minimum detectable concentration (MDC) of IFN-γ is typically less than 20 pg/mL. The MDC was determined by adding two standard deviations to the mean optical density value of twenty zero standard replicates and calculating the corresponding concentration.

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2. Linearity

Three samples (Serum) spiked with high concentrations of IFN-γ were serially diluted with dilution buffer to produce samples with values within the dynamic range of the assay and then assayed. The average recovery of IFN-γ for serum samples is 101.4%.
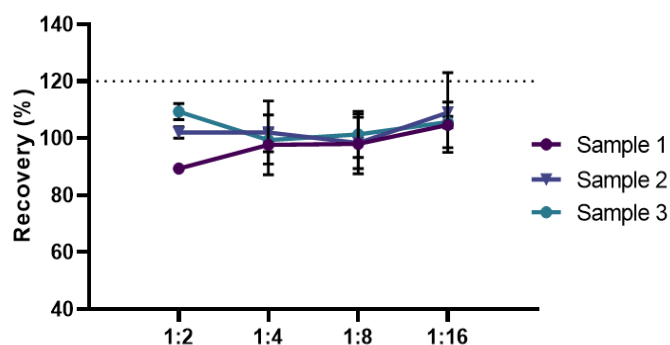
3. Intra-Assay Precision

Ten replicates of each of 3 samples containing different IFN-γ concentrations were tested in one assay. Acceptable criteria: CV < 10%.

Sample Concentration (pg/mL)	Mean (pg/mL)	SD	Numbers	CV (%)
2000	2004.31	68.53	10	3.4
500	502.16	17.32	10	3.5
100	117.45	7.45	10	6.4

4. Inter-Assay Precision

3 samples containing different concentrations of IFN-γ were tested in independent assays. Acceptable criteria: CV<15%.

Sample Concentration (pg/mL)	Mean (pg/mL)	SD	Numbers	CV (%)
2000	2024.30	92.19	9	4.6
500	504.52	24.06	9	4.8
100	106.92	9.03	9	8.4

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## 5. Recovery

Recombinant IFN-γ was spiked into 3 human serum samples, and then analyzed. The average recovery of IFN-γ for serum samples is 108.9%.

| Sample ID	Conc Measured (pg/mL)	Conc Added (pg/mL)	Conc Recovered (pg/mL)	Recovery (%)
1	1931.75	1750	1931.75	110.4
	918.41	875	918.41	105.0
	416.83	437.5	416.83	95.3
	239.18	218.8	239.18	109.3
	-	-		
2	1934.91	1750	1934.91	110.6
	975.52	875	975.52	111.5
	454.98	437.5	454.98	104.0
	244.74	218.8	244.74	111.9
	-	-		
3	1967.39	1750	1967.39	112.4
	953.65	875	953.65	109.0
	462.23	437.5	462.23	105.7
	233.64	218.8	233.64	106.8
	-	-		

6. Hook Effect

Not be affected by the concentration of IFN-γ up to 10 ng/mL.
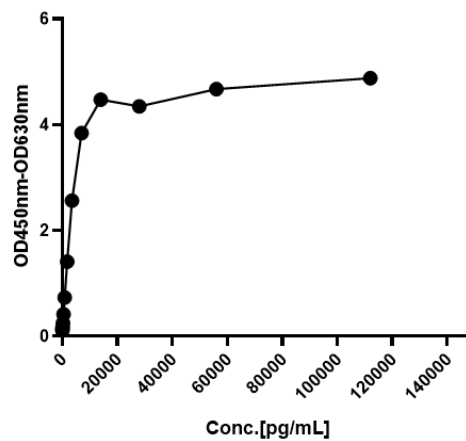
7. Sample Values

40 healthy serum samples were evaluated for the concentrations of human IFN-γ in assay.
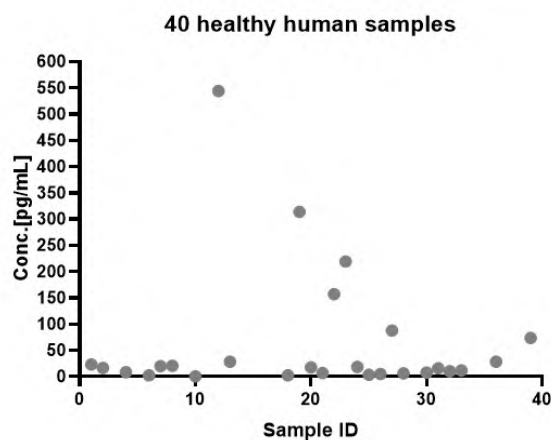
Human peripheral blood mononuclear cells (PBMCs) obtained were plated at a density of 1×10^6 cells/mL in complete RPMI 1640 medium containing 10% fetal bovine serum, 2 mM L-glutamine, and antibiotic supplements (penicillin 100 U/mL with streptomycin sulfate 100 µg/mL). Following a 5-day incubation period, experimental groups were either maintained as untreated controls or stimulated with 10 µg/mL phytohemagglutinin (PHA). Subsequent analysis involved quantitative measurement of IFN-gamma concentrations in collected culture supernatants using standardized immunoassays.

Condition	Day 3 Mean (pg/mL)	Day 5 Mean (pg/mL)
Unstimulated	ND	ND
Stimulated with PHA	67,385	125,006

ND=Non-detectable

TROUBLESHOOTING GUIDE

Problem	Cause	Solution
Poor standard curve	* Inaccurate pipetting	* Check pipettes
Large CV	* Inaccurate pipetting * Air bubbles in wells	* Check pipettes * Remove bubbles in wells
High background	* Plate is insufficiently washed * Contaminated wash buffer	* Review the manual for proper wash. * Make fresh wash buffer
Very low readings across the plate	* Incorrect wavelengths * Insufficient development time	* Check filters/reader * Increase development time
Samples are reading too high, but standard curve looks fine	* Samples contain cytokine levels above assay range	* Dilute samples and run again
Drift	* Interrupted assay set-up * Reagents not at room temperature	* Assay set-up should be continuous - have all standards and samples prepared appropriately before commencement of the assay * Ensure that all reagents are at room temperature before pipetting into the wells unless otherwise instructed in the antibody inserts