D001-EN.01

Human Interferon-γ (IFN-γ) ELISA Assay Pair

(Enzyme-Linked Immunosorbent Assay)

Catalog Number：CRS-D001

Pack Size: 5 plates

IMPORTANT: Please carefully read this manual before performing your experiment.

For Research Use Only. Not For Use in Diagnostic or Therapeutic Procedures

INTENDED USE

The kit is developed for quantitative detection of IFN-γ in human serum and cell culture supernates. It is intended for research use only (RUO).

BACKGROUND

Interferon gamma (IFN-γ), one of the most important biomarkers of CRS, is secreted by CD8+ T cells, Th1 CD4+ T and natural killer cells (NK) under various stimuli. By inhibiting Th2 cell differentiation, stimulating Th1 cell proliferation, promoting macrophage activation and inducing MHC I/MHC II expression, it achieves a wide range of biological functions such as antiviral, immune regulation and regulation of cell proliferation and differentiation.

To support the development of CAR-T drugs, ACROBiosystems independently developed human Interferon-γ (IFN-γ) ELISA Assay Pair, which is used for detection and evaluation stimulatory effects of T cell activating agents for evaluation the efficacy and function of CAR-T products in drug development and CMC quality control stages.

PRINCIPLE OF THE ASSAY

This assay kit is used to measure the levels of human Interferon-γ (IFN-γ) by employing a standard sandwich-ELISA format. Firstly, attach the Human IFN-γ Capture Antibody to the microplate, add the standard samples provided in kit and your samples to the plate, incubate and wash the wells. Then add the Human IFN-γ Detection Antibody to the plate and form Antibody-antigen-biotinylated antibody complex, incubate and wash the wells. Next add Streptavidin-HRP to the plate, incubate and wash the wells. At last, load the substrate into the wells and monitor solution color from blue to yellow. The reaction is stopped by the addition of a stop solution and the intensity of the absorbance can be measured at 450nm and 630nm. The OD Value reflects the amount of IFN-γ bound.

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PRECAUTIONS

1. This kit is for research use only and is not for use in diagnostic or therapeutic applications.
2. The kit is suitable for cell supernatant, serum and plasma samples.
3. Do not use reagents past their expiration date.
4. Do not mix or substitute reagents with those from other kits or other lot number kits.
5. If samples generate values higher than the highest standard, dilute the samples with the appropriate calibrator diluent and repeat the assay. If cell supernatant samples need step dilution, except for the final dilution with diluent, other intermediate dilutions can be in cell culture medium.
6. Differences in test results can be caused by a variety of factors, including laboratory operator, pipette usage, plate washing technique, reaction time or temperature, and kit storage.
7. This kit is designed to remove or reduce some endogenous interference factors in biological samples, and not all possible influencing factors have been removed.

MATERIALS PROVIDED

Table1. Materials provided

Catalog	Components	Size (5 plates)	Format	Storage	
				Unopened	Opened
CRD001-C01	Human IFN-γ Capture Antibody	30 μg	Powder	2-8°C	-70°C
CRD001-C02	Human IFN-γ Standard	30 μg	Powder	2-8°C	-70°C
CRD001-C03	Human IFN-γ Detection Antibody	60 μg	Powder	2-8°C	-70°C
CRD001-C04	Streptavidin-HRP	100 μL	Liquid	2-8°C, avoid light	2-8°C, avoid light

SRORAGE

1. The unopened kit is stable for 12 months from the date of manufacture if stored at 2°C to 8°C.
2. The opened kit should be stored per Table 1. The shelf life is 30 days from the date of opening.

Note: a. Do not use reagents past their expiration date.

b. Find the expiration date on the outside packaging.

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OTHER MATERIALS & SOLUTIONS REQUIRED

1. 96 well microplates：Corning 、Catalog# 42592
2. Coating Buffer (1xPBS)：Solarbio、Catalog # P1020 (1.5 mM KH_2PO_4、8.1 mM Na_2HPO_4、137 mM NaCl、2.7 mM KCl，pH 7.2-7.4，0.2 μm filtered)
3. 1xWashing Buffer(1xPBST)：Solarbio、Catalog # P1033 (0.05% Tween-20 in PBS，pH 7.2-7.4)
4. Blocking Buffer：2% BSA (Yancheng Saibao、Catalog # N/A) in 1xWashing Buffer
5. Dilution Buffer：0.5% BSA (Yancheng Saibao、Catalog # N/A) in 1xWashing Buffer
6. Substrate Solution：InnoReagents、Catalog # TMB-S-004
7. Stop Solution：2 N H_2SO4

REAGENT PREPARATION

Bring all reagents and samples to room temperature (20℃-25℃) before use. If crystals have formed in buffer solution, place the sample in an 37℃ incubator until the crystals have completely dissolved and bring the solution back to room temperature before use.

According to Table 2, prepare the provided lyophilized product into a storage solution with ultrapure water, dissolve at room temperature for 15 to 30 minutes, and mix by gently pipetting, avoiding vigorous shaking or vortexing. The reconstituted storage solution should be stored at -70°C. It is recommended that the number of freezing and thawing should not exceed 1 time, and the size of the aliquot should not be less than 10 μg.

Table 2. Preparation method

ID	Components	Size (5 plates)	Storage solution concentration.	Reconstituted water Vol.
CRD001-C01	Human IFN-γ Capture Antibody	30 μg	200 μg/mL	150 μL
CRD001-C02	Human IFN-γ Standard	30 μg	150 μg/mL	200 μL
CRD001-C03	Human IFN-γ Detection Antibody	60 μg	200 μg/mL	300 μL

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RECOMMENDED SAMPLE PREPARATION

1. Coating

Dilute Human IFN-γ Capture Antibody stock solution (200 μg/mL) to 0.5 μg/mL with Coating Buffer to make Human IFN-γ Capture Antibody working solution. Add 100 μL of Human IFN-γ Capture Antibody working solution (0.5 μg/mL) to each well, seal the plate with microplate sealing film and incubate overnight (or 16 hours) at 4℃.

2. Washing

Remove the remaining solution by aspiration, add 300 μL of 1×Washing Buffer to each well, gently tap the plate for 1 minute, remove any remaining 1×Washing Buffer by aspirating or decanting, invert the plate and blot it against paper towels. Repeat the wash step above for three times.

3. Blocking

Add 300 μL Blocking Buffer to each well, seal the plate with microplate sealing film and incubate at room temperature for 2.0 hours.

4. Washing

Repeat step 2.

5. Add Standard and Samples

5.1 Preparation of Standard curve

The concentration of the reconstituted human IFN-γ Calibrator (CRD001-C02) is 150 μg/mL，prepare (Std.-0) by diluting 5 μL the reconstituted human IFN-γ Calibrator into 245 μL Sample Dilution Buffer, mix gently well. Then prepare Std.- 1' by diluting 10 μL Std.-0 into 290 μL Sample Dilution Buffer. At last, prepare the highest concentration of standard curve, **Std.-1 (1250 pg/mL)**, by diluting 10 μL Std.- 1' into 790 μL Sample Dilution Buffer. Prepare 1:1 serial dilutions for the standard curve as follows: Pipette 300 μL of Sample Dilution Buffer into each tube. Make sure to mix well every time. Sample Dilution Buffer serves as blank.

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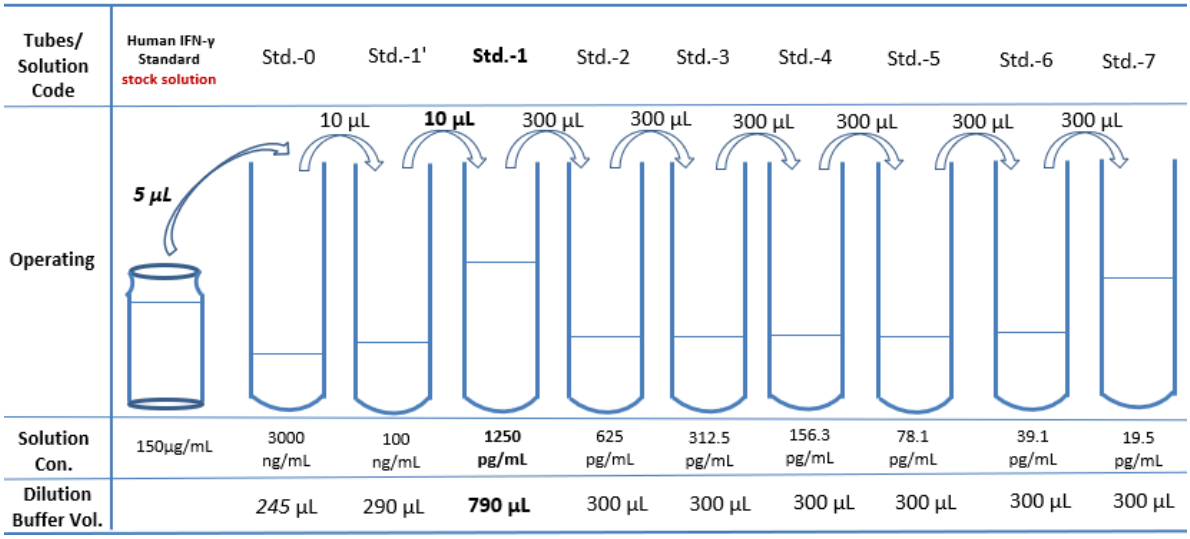

5.2 Preparation of Samples

a. If the sample to be tested is the serum or plasma, dilute test sample at 1:5 with Dilution Buffer. The volume ratio of sample to diluent is 1:4.

b. If the sample to be tested is the cell supernatant, dilute test sample at 1:2 with Dilution Buffer. The volume ratio of sample to diluent is 1:1.

5.3 Add Samples

Add 100 µL Standard (Std.-1 ~ Std.-7) and Samples to each well. For blank Control wells, please add 100 µL Dilution Buffer.

Note: It is recommended to set doeble holes for samples and standard curves to be tested.

6. Incubation

Seal the plate with microplate sealing film and incubate at room temperature for 1 hour.

7. Washing

Repeat step 2.

8. Add Human IFN-γ Detection Antibody

Dilute Biotinylated-Human IFN-γ Detection Antibody stock solution (200 µg/mL) to 1.0 µg/mL with Dilution Buffer to make Biotinylated-Human IFN-γ Detection Antibody working solution. For all wells, add 100 µL Biotinylated-Human IFN-γ Detection Antibody (1.0 µg/mL) working solution. Please prepare it for one-time use only.

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9. Incubation

Seal the plate with microplate sealing film and incubate at room temperature for 1 hour.

10. Washing

Repeat step 2.

11. Add Streptavidin-HRP

For all wells, add 100 µL Streptavidin-HRP (dilute at 1:2000) working solution. Please prepare it for one-time use only, avoid light.

12. Incubation

Seal the plate with microplate sealing film and incubate at room temperature for 30 min.

13. Washing

Repeat step 2.

14. Substrate Reaction

Add 100 µL Substrate Solution to each well. Seal the plate with microplate sealing film and incubate at room temperature for 20 min, avoid light.

15. Termination

Add 50 µL Stop Solution to each well, and tap the plate gently to allow thorough mixing.

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

16. Data Recording

Read the absorbance at 450 nm and 630 nm using UV/Vis microplate spectrophotometer within 10 minutes.

Note: To reduce the background noise, subtract the value read at OD450nm with the value read at OD630 nm.

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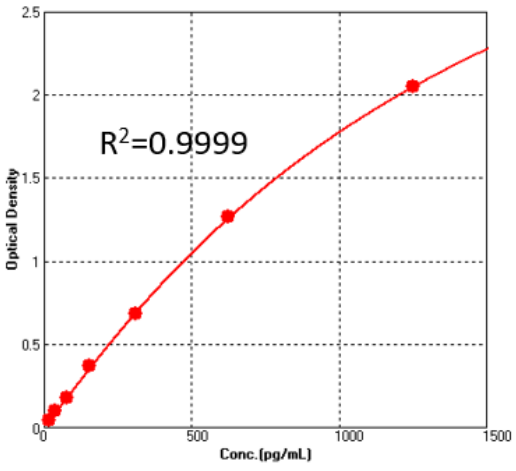
CALCULATION OF RESULTS

1. Calculate the mean absorbance for each standard, control and sample and subtract average zero standard optical density (O.D.).
2. The standard curve is plotted with the standard concentration as x-axis and the calibrated absorbance value as y-axis. Four parameters logistic are used to draw the standard curve and calculate the sample concentration.
3. Normal range of Standard curve: R2≥0.9900.
4. Detection range: 19.5 pg/mL-1250 pg/mL. If the OD value of the sample to be tested is higher than 1250 pg/mL, the sample shall be diluted with dilution buffer and assay repeated. If the OD value of the sample to be tested is lower than 19.5 pg/mL, the sample should be reported.

TYPICAL DATA

The following data is for reference only. The sample concentration was calculated based on the results of the standard curve.



Conc.(pg/mL)	O.D.-1	O.D.-2	Average	Corrected
1250	2.054	2.133	2.094	2.050
625	1.383	1.243	1.313	1.270
312.5	0.725	0.738	0.732	0.688
156.25	0.449	0.384	0.417	0.373
78.125	0.239	0.214	0.227	0.183
39.0625	0.146	0.142	0.144	0.101
19.53125	0.093	0.089	0.091	0.048
0	0.044	0.043	0.044	/

SPECIFICITY

This assay recognizes natural and recombinant human IFN-γ. No cross-reactivity was observed when this kit was used to analyze the following recombinant cytokines.

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Human	
IL-2	IL-7
IL-4	TNF-α
IL-6	IL-1β
IL-10	IL-15
GM-CSF	IL-21

CALIBRATION

This immunoassay is calibrated against a highly purified E. coli-expressed recombinant human IFN-γ (87/586). Reference Reagent is calibrated by NIBSC/WHO in April 2013.

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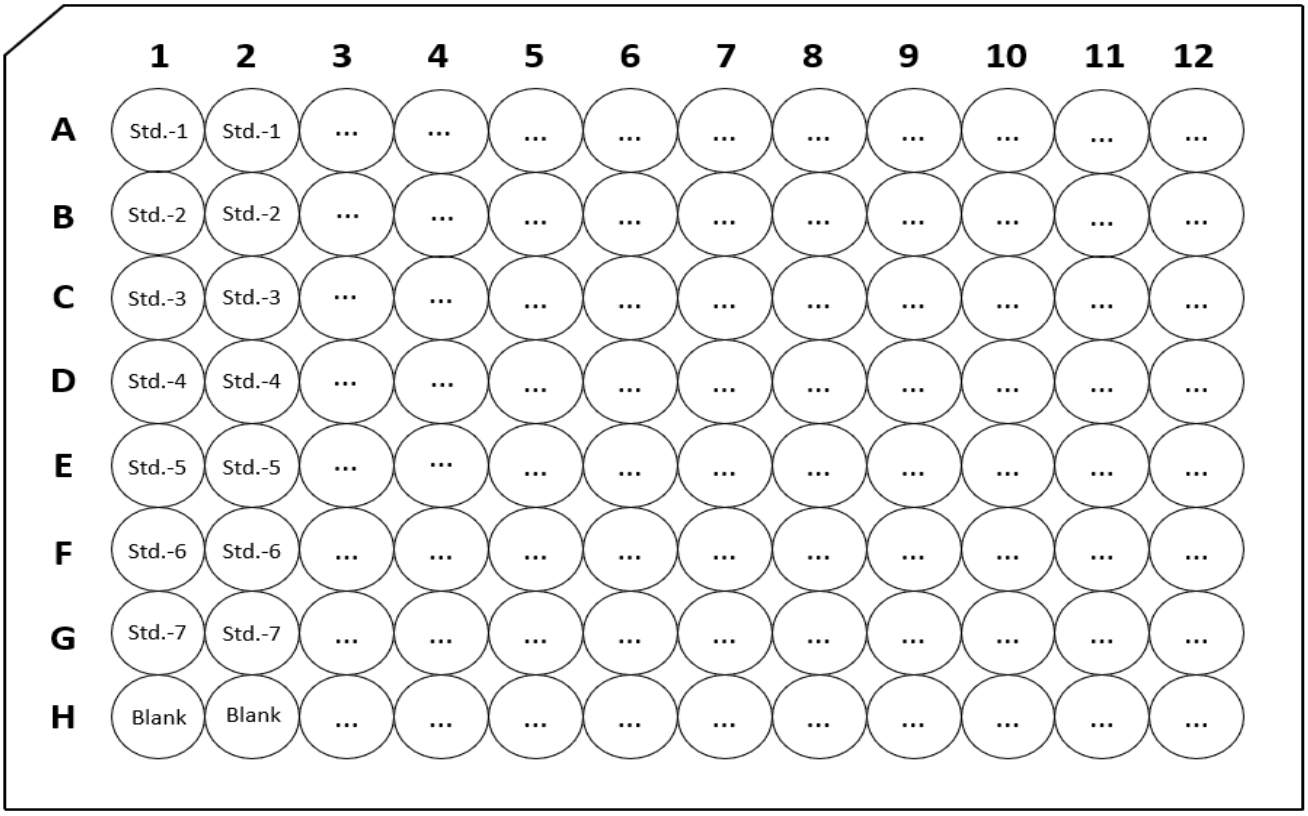
PLATE LAYOUT



Note: Blank is a Blank Dilution Buffer hole.

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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

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