Human Interferon-γ (IFN-γ) ELISA Kit

(Enzyme-Linked Immunosorbent Assay)

Catalog Number：CRS-A017

Pack Size: 96 tests

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 mAbs CAR-T drugs, ACROBiosystems independently developed human Interferon-γ (IFN-γ) ELISA quantitative detection kit via rigorous methodological validation, 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. The micro-plate in the kit has been pre-coated with Anti-IFN-γ Antibody. Firstly, add the standard samples provided in kit and your samples to the plate, incubate and wash the wells. Then add the Biotin-Anti-IFN-γ 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.

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 (96 tests)	Format	Storage	
				Unopened	**Opened**
CRS017-C01	Pre-coated Anti-IFN-γ Antibody Microplate	1 plate	Solid	2-8°C	2-8°C
CRS017-C02	Human IFN-γ Standard	30 μg	Powder	2-8°C	-70°C
CRS017-C03	Biotin-Anti- IFN-γ Antibody	25 μg	Powder	2-8°C	-70°C
CRS017-C04	Streptavidin-HRP	50 μL	Liquid	2-8°C, avoid light	2-8°C, avoid light
CRS017-C05	10xWashing Buffer	50 mL	Liquid	2-8°C	2-8°C
CRS017-C06	2xDilution Buffer	50 mL	Liquid	2-8°C	2-8°C
CRS017-C07	Substrate Solution	12 mL	Liquid	2-8°C, avoid light	2-8°C, avoid light
CRS017-C08	Stop Solution	7 mL	Liquid	2-8°C	2-8°C

NOTE: Bubbles in microplate wells do not affect the experiment and require no action. Proceed with the experimental procedures and methods described below.

SRORAGE

1. The unopened kit is stable for 24 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.

REAGENTS/EQUIPMENT NEEDED BUT NOT SUPPLIED

Single or multi-channel micropipettes and pipette tips: need to meet 10 μL, 300 μL, 1000 μL injection; requirements;

37° C Incubator;

Single or dual wavelength microplate reader with 450nm and 630nm filter;

Tubes: 1.5mL,10mL;

Timer;

Reagent bottle;

Deionized or distilled water.

REAGENT PREPARATION

Bring all reagents and samples to room temperature (20°C-25°C) before use. If crystals have formed in buffer solution, place the sample in an 37°C 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 (96 T)	Storage solution concentration.	Reconstituted water Vol.
CRS017-C02	Human IFN-γ Standard	30 μg	200 μg/mL	150 μL
CRS017-C03	Biotin-Anti- IFN-γ Antibody	25 μg	200 μg/mL	125 μL

RECOMMENDED SAMPLE PREPARATION

1. Working Solution Preparation

1.1 Preparation of 1×Washing Buffer:

Dilute 50 mL 10×Washing Buffer with ultrapure water/deionized water to 500 mL.

1.2 Preparation of 1×Dilution Buffer:

Dilute 50 mL 2×Dilution Buffer with 1×Washing Buffer to 100 mL.

1.3 Preparation of Biotin-Anti-IFN-γ Antibody working fluid:

Dilute Biotin-Anti-IFN-γ Antibody with 1×Dilution Buffer to 1.0 μg/mL. Please prepare it for one-time use only.

1.4 Preparation of Streptavidin-HRP working fluid:

Dilute Streptavidin-HRP at 1:2000 with 1×Dilution Buffer. The prepared working fluid should avoid light. Please prepare it for one-time use only.
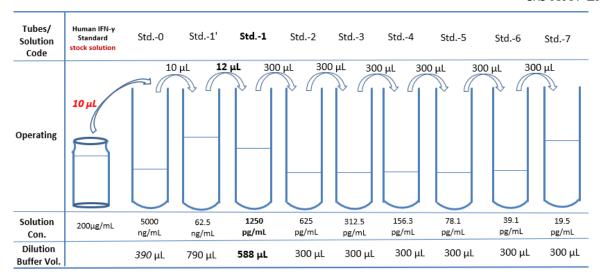
1.5 Sample preparation

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

2. Preparation of Standard curve

The concentration of the reconstituted human IFN-γ Standard (CRS017-C02) is 200 μg/mL，prepare (Std.-0) by diluting 10 μL the reconstituted human IFN-γ Standard into 390 μL Sample Dilution Buffer, mix gently well. Then prepare Std.- 1' by diluting 10 μL Std.-0 into 790 μL Sample Dilution Buffer. At last, prepare the highest concentration of standard curve, **Std.-1 (1250 pg/mL)**, by diluting 12 μL Std.- 1' into 588 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.

Tubes/ Solution Code	Human IFN-γ Standard stock solution	Std.-0	Std.-1'	Std.-1	Std.-2	Std.-3	Std.-4	Std.-5	Std.-6	Std.-7
Operating	10 µL	10 µL	12 µL	300 µL	300 µL	300 µL	300 µL	300 µL	300 µL	
Solution Con.	200µg/mL	5000 ng/mL	62.5 ng/mL	1250 pg/mL	625 pg/mL	312.5 pg/mL	156.3 pg/mL	78.1 pg/mL	39.1 pg/mL	19.5 pg/mL
Dilution Buffer Vol.		390 µL	790 µL	588 µL	300 µL	300 µL	300 µL	300 µL	300 µL	300 µL

3. Add Samples

Add 100 µL Calibrator 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.

4. Incubation

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

5. Washing

Remove the remaining solution by aspiration, add 300 µL of 1×Washing Buffer to each well, soak for 10s, 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.

6. Add Biotin-Anti-IFN-γ Antibody

For all wells, add 100 µL Biotin- Anti-IFN-γ Antibody (1.0 µg/mL) working solution. Please prepare it for one-time use only.

7. Incubation

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

8. Washing

Repeat step 5.

9. 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.

10. Incubation

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

11. Washing

Repeat step 5.

12. 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.

13. 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.

14. 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 readings at 630nm from the readings at 450nm.

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: $R2 \geq 0.9900$.

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.
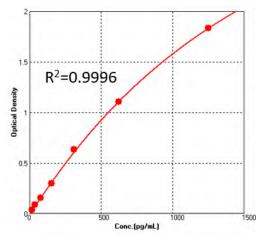
QUICK GUILD

1 Working fluid perparation and preparation of Standard curve

↓

2 Add 100µl standard, sample and blanks to wells

↓ 18-25℃ 1.0 hour
Aspirate wells & Wash×3

3 Add 100µl Biotin-Anti-IFN-γ Antibody working solution

↓ 18-25℃ 1.0 hour
Aspirate wells & Wash×3

4 Add 100µl Streptavidin-HRP working solution

↓ 18-25℃ 30 min
Aspirate wells & Wash×3

5 Add 100µl of Substrate Solution

↓ 18-25℃ 20 min
Avoid light

6 Add 50µl Stop Solution and a colorimetric change occurs

↓ <10 min

7 Read absorbance

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	1.889	1.886	1.888	1.835
625	1.102	1.210	1.156	1.104
312.5	0.688	0.683	0.686	0.633
156.25	0.347	0.356	0.352	0.299
78.125	0.213	0.201	0.207	0.155
39.0625	0.126	0.158	0.142	0.090
19.53125	0.093	0.082	0.088	0.035
0	0.055	0.049	0.052	/

SENSITIVITY

The minimum detectable concentration of human IFN-γ is 5.309 pg/mL. The minimum detectable concentration was determined by adding twice standard deviations to the mean optical density value of twenty zero standard replicates and calculating the corresponding concentration.

PRECISION

1. Intra-assay Precision

Three samples of known concentration were tested twenty times on one plate to assess intra-assay precision.
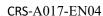
2. Inter-assay Precision

Three samples of known concentration were tested on 3 different plates, 10 replicates in each plate to assess inter-assay precision.

	Intra-assay Precision			Inter-assay Precision		
Sample	1	2	3	1	2	3
n	20	20	20	30	30	30
Mean (pg/mL)	965.508	490.722	245.491	969.837	500.442	251.666
SD	62.455	16.545	3.843	16.377	8.873	13.419
CV (%)	6.5%	3.4%	1.6%	1.7%	1.8%	5.3%

Note: *The example data is for reference only.*

RECOVERY

Three parts of blank serum were added with different concentrations of human IFN-γ, and the serum without human IFN-γ was used as background to calculate the recovery rate. The range of the recovery rate is 85.2-105.2%, and the average recovery is 93.5%.

Sample Type	Average % Recovery	Range
Serum(n=5)	93.5	85.2-105.2%

LINEARITY

To assess the linearity of the assay, samples spiked with high concentrations of human IFN-γ were serially diluted with calibrator diluent to produce samples with values within the dynamic range of the assay.

		Cell culture medium	Serum	Citrate plasma
1:2	Average Recovery (%)	92.6	107.7	96.1
	Range (%)	88.9-95.6	103.7-111.3	91.1-99.9
1:4	Average Recovery (%)	99.6	109.6	94.6
	Range (%)	98.6-101.1	100.2-116.3	92.9-95.6
1:8	Average Recovery (%)	91.1	94.6	95.7
	Range (%)	88.5-94.3	88.4-106.4	93.5-98.0

Note: *The example data is for reference only.*

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.

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.

NIBSC/WHO (87/586) approximate value (U/mL) = 0.007 × Human IFN-γ value (pg/mL)

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Asia and Pacific:	Tel: +86 400-682-2521	E-mail: order@acrobiosystems.com

PLATE LAYOUT

	1	2	3	4	5	6	7	8	9	10	11	12
A	Std.-1	Std.-1	...	...	...	...	...	...	...	...	...	...
B	Std.-2	Std.-2	...	...	...	...	...	...	...	...	...	...
C	Std.-3	Std.-3	...	...	...	...	...	...	...	...	...	...
D	Std.-4	Std.-4	...	...	...	...	...	...	...	...	...	...
E	Std.-5	Std.-5	...	...	...	...	...	...	...	...	...	...
F	Std.-6	Std.-6	...	...	...	...	...	...	...	...	...	...
G	Std.-7	Std.-7	...	...	...	...	...	...	...	...	...	...
H	Blank	Blank	...	...	...	...	...	...	...	...	...	...

Note: Blank is a Blank Dilution Buffer hole.

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