NEED HELP?

User Manual

Disclaimer: Products are intended for research use only

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Human IFN-γ ELISA Kit

User Guide

PLEASE READ THE DOCUMENT CAREFULLY BEFORE EXPERIMENT

Product No.: 1402430
Version: A/0
For Research Use Only

Biofargo, Inc.

■ Product Name

Human IFN-γ ELISA Kit

■ Package

96 tests/Kit

■ Intended Use

This kit is suitable for quantitation of Human Interferon Gamma (IFN-γ), such as potency assay of CAR-T cells.

The kit is for RESEARCH USE ONLY and not intended for clinical use.

■ Product Description

This kit is based on the solid-phase Enzyme-linked Immunosorbent Assay (ELISA) with a double-antibody sandwich technique to detect Human IFN-γ. The antibody specific to Human IFN-γ was employed in the assay to capture any Human IFN-γ in the sample. Both the calibration standards and test samples were simultaneously added to the microtiter plate coated with the affinity purified capture antibody, and followed by incubation and washing. The biotinylated antibody was added to the microtiter plate to bind the Human IFN-γ and then reacted with streptavidin labeled HRP (Horseradish Peroxidase). TMB (3,3',5,5'-tetramethylbenzidine) substrate was added into reaction, HRP catalyzed the oxidation of TMB by H_2O_2 to produce a blue colored product (maximum absorption peak at 655 nm). Then the stop solution was added to terminate the enzymatic reaction, resulting in a yellow colored product (maximum absorption peak at 450 nm). The absorbance values at 450 nm wavelength were positively correlated with the Human IFN-γ concentration in the calibration standard and the samples. The concentration of Human IFN-γ in the samples can be calculated using a dose-response curve.

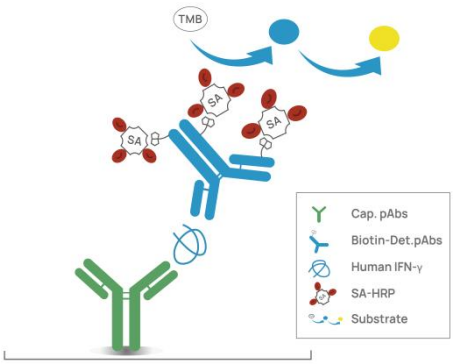


Figure 1. Schematic diagram

■ Kit Contents

Table 1. Kit Components

Reagent	Part No.	Quantity	Note
Human IFN-γ Calibration Standard	PNB023	3 bottles	Lyophilized powder. Dissolve it with the Reconstitution Solution (300 μL), and let it stand for about 5 minutes until transparent. Please refer to the bottle label for details.
Anti-Human IFN-γ Microtiter Strips	PNA018	8 well × 12 strips	Strips pre-coated with anti-Human IFN-γ antibody in a vacuumed bag with desiccant. Seal and store immediately after use.
Reconstitution Solution	PNC002	1 × 1.5 mL	Only used for dissolving Human IFN-γ Calibration Standard.
Diluent	PNE004	2 × 25 mL	For dilution of Calibration Standard, Streptavidin-HRP(100×), Anti-Human IFN-γ: Biotinylated Conjugate (100×) and samples.
Wash Buffer Concentrate (10×)	PNF001	2 × 25 mL	For plate washing. Dilute 10 times with freshly prepared ultra-pure water to obtain 1×Wash Buffer.
Anti-Human IFN-γ: Biotinylated Conjugate (100×)	PNG012	1 × 120 μL	Biotinylated anti-Human IFN-γ antibody. Seal and protect from light. Dilute 100 times with Diluent before use.
Streptavidin-HRP (100×)	PNH002	1 × 140 μL	Streptavidin labeled with HRP. Seal and protect from light. Dilute 100 times with Diluent before use.
TMB Substrate	PND004	1 × 12 mL	Seal and protect from light. Equilibrate to room temperature (RT) for 20 minutes before use.
Stop Solution	PNI002	1 × 6 mL	1 M hydrochloric acid. Avoid direct contact with eyes, skin, and clothing. Wear goggles while handling.
Sealing Film	PNK001	3 pieces	Cover the strips during incubation to prevent contamination and liquid evaporation.

Note: Room temperature refers to 25 ± 3℃.

■ Storage Conditions

Store the kit at 2-8℃. Please check the expiration date on the labels. The opened components should be stored as shown in Table 2.

Table 2. Recommended storage conditions for opened components

Component	Stability
Anti-Human IFN-γ Microtiter Strips	Store in the bag with desiccant at 2-8℃ for up to 90 days.
Dissolved Calibration Standards	For single use only.

■ Materials Required But Not Provided

- Sterile centrifuge tubes for dilution
- Absorbent paper for plate drying
- Pipette Tips: 1000 μL, 100 μL and 10 μL
- Multi-channel reagent reservoirs (50 mL)

■ Equipment

- Microplate reader capable of measuring absorbance at 450 nm, with the correction wavelength set at 620 nm to 650 nm.
- Single or multi-channel Pipettes: 1000 μL, 100 μL and 10 μL
- Microplate thermoshake
- Incubator (optional)
- Plate washer (optional)

■ Workflow

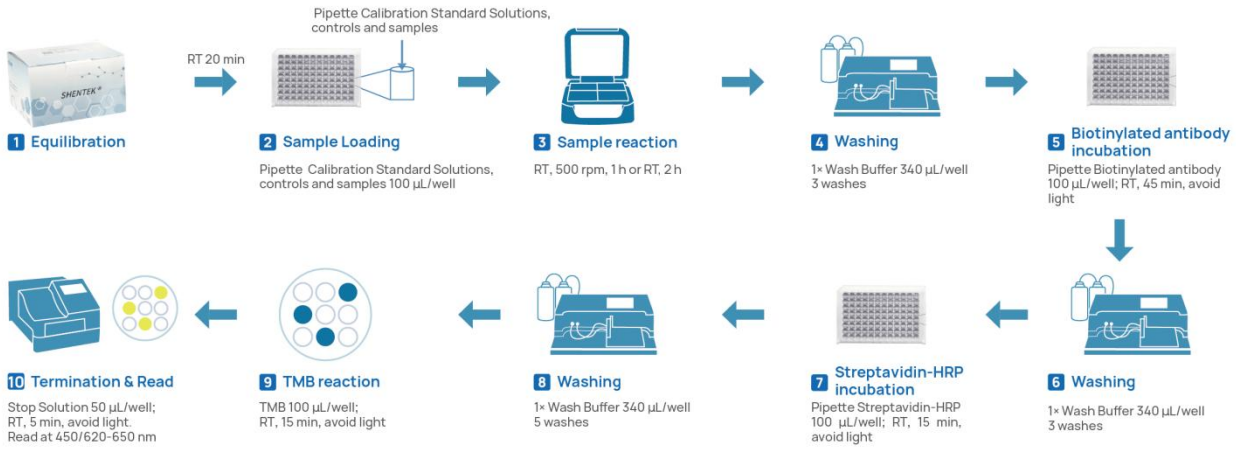


Figure 2. Procedure flowchart

1. Preparation

(1) Equilibration

- Allow the kit to equilibrate at room temperature for 20 minutes before use. Return to 2-8℃ immediately after use.
- Take appropriate amount of strips to a strip holder according to the experimental design. Please store the remaining strips in the bag with desiccant at 2-8℃.

(2) Preparation of Reagents

- Calibration Standard Solution: Pipette 300 µL of Reconstitution Solution into the bottle containing Human IFN-γ Calibration Standard. Gently invert to mix well and let it stand for 5 minutes.

  Note: Do not use other volumes of Reconstitution Solution to dissolve the Calibration standard.
- 1×Wash Buffer: Dilute 1 volume of Wash Buffer Concentrate (10×) with 9 volumes of ultra-pure water. For example, add 25 mL Wash Buffer Concentrate (10×) to 225 mL of ultra-pure water to make 250 mL of 1×Wash Buffer. Mix well before use.

  Note: If the Wash Buffer Concentrate (10×) or Diluent is cloudy or contains precipitates, heat at 37℃ until it clears.

- 1×Anti-Human IFN-γ:Biotinylated Conjugate: Dilute the Anti-Human IFN-γ:Biotinylated Conjugate (100×) with Diluent in a new centrifuge tube to prepare the 1×Anti-Human IFN-γ: Biotinylated Conjugate, mix gently and prepare it freshly.
- 1×Streptavidin-HRP: Dilute the Streptavidin-HRP (100×) with Diluent in a sterile centrifuge tube to prepare the 1×Streptavidin-HRP, mix gently and prepare it freshly.

(3) Preparation of Calibration Standard Solutions

- Prepare Calibration Standard Solutions as indicated in Fig 3 and Table 3.

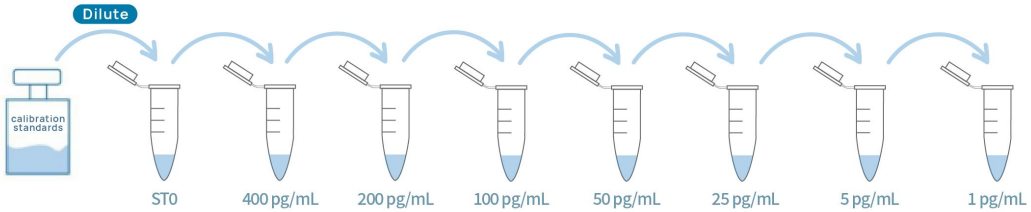


Figure 3. Graphic scheme of Calibration Standard Solutions preparation

Table 3. Preparation of Calibration Standard Solutions

Tubes	Dilution procedure	Conc. (pg/mL)
ST0*	50 µL reconstituted Calibration Standard + 450 µL Diluent	/
ST1	Dilute ST0 with Diluent to ST1	400
ST2	500 µL ST1+ 500 µL Diluent	200
ST3	500 µL ST2 + 500 µL Diluent	100
ST4	500 µL ST3 + 500 µL Diluent	50
ST5	500 µL ST4 + 500 µL Diluent	25
ST6	200 µL ST5 + 800 µL Diluent	5
ST7**	200 µL ST6 + 800 µL Diluent	1
NCS	Diluent	0

*ST0 can be stabilized at -65℃ for 15 days, avoid repeated freeze-thaw cycles.

**Anchor point

(4) Sample Preparation

- Test samples: Cell culture supernatants, or others. Make sure samples are clear and transparent, and insoluble substances need to be removed by centrifugation or filtration.

- Conduct sample stability studies to prevent degradation or denaturation during the experiment. Avoid repeated freeze-thaw cycles. For long-term storage, -65°C or below is recommended to avoid degradation.
- Dilute the samples with a suitable diluent to achieve a proper range of Human IFN-γ concentration within the calibration curve.
- For the first use, a method validation is recommended to verify sample suitability before the subsequent routine test. This will help to set up appropriate sample dilution series.

  Note: Please contact us for support of validation protocol.

2. Assay Experiment

(1) Sample Incubation

- Pipette 100 μL of Calibration Standard Solutions, NCS (Diluent) and samples into each designated well according to the experimental design. Avoid foaming bubbles during pipetting. We recommend to prepare 2-3 replicates for each concentration.
- Seal the plate and incubate on microplate thermoshaker at 500 rpm for 1 hour at room temperature. (If not equipped with a microplate thermoshake, 2-hour static incubation at room temperature is optional).

Table 4. Example of the 96 tests layout

	1	2	3	4	5	6	7	8	9	10	11	12
A	NCS	NCS	NCS		S1	S1	S1					
B	ST7	ST7	ST7		S2	S2	S2					
C	ST6	ST6	ST6		S3	S3	S3					
D	ST5	ST5	ST5									
E	ST4	ST4	ST4		S1+SRC	S1+SRC	S1+SRC					
F	ST3	ST3	ST3		S2+SRC	S2+SRC	S2+SRC					
G	ST2	ST2	ST2		S3+SRC	S3+SRC	S3+SRC					
H	ST1	ST1	ST1									

✧ “ST1-ST7” indicate 7 concentration gradients, “NCS” as negative control, “S1-S3” as test samples and “S1 SRC-S3 SRC” as the spiked recovery controls for each sample.

✧ The number of replicates and the spiked samples can be determined by method validation.

(2) Biotinylated Antibody Incubation

- Wash the plate with 340 μL of 1×Wash Buffer per well. Wipe off any liquid from the bottom outside of the plate. Repeat washing for 3 times. Do not allow the wells to be completely dried before adding the substrate.
- Pipette 100 μL of 1×Anti-Human IFN-γ:Biotinylated Conjugate into the corresponding wells as indicated earlier.
- Seal the plate and incubate for 45 minutes at room temperature, and protect from light.

(3) Streptavidin-HRP Incubation

- Wash the plate with 340 μL of 1×Wash Buffer per well. Wipe off any liquid from the bottom outside of the plate. Repeat washing for 3 times. Do not allow the wells to be completely dried before adding the substrate.
- Pipette 100 μL of 1×Streptavidin-HRP into the corresponding wells.
- Seal the plate and incubate for 15 minutes at room temperature, and protect from light.

(4) TMB Reaction

- Equilibrate the TMB Substrate for 20 minutes at room temperature.
- Wash the plate with 340 μL of 1×Wash Buffer per well. Wipe off any liquid from the bottom outside of the plate. Repeat washing for 5 times. Do not allow the wells to be completely dried before adding the substrate.
- Add 100 μL of TMB Substrate into the wells, and incubate at room temperature for 15 minutes, and protect from light.

  Note: Do not use sealing film during this step.

(5) Termination

- Add 50 μL of Stop Solution into each well.

  Note: The order of adding Stop Solution should be the same as the order of adding the TMB Substrate. While adding samples, suspend the tips above the liquid to prevent contact with the solution in the wells and minimize the risk of bubble formation.
- Incubate at room temperature for 5 minutes, protect from light.

(6) Reading

- Read absorbance at 450 nm/620-650 nm.

3. Calculation and Analysis

- The OD_{450nm} value of each well should be calculated by subtracting their respective long wavelength, as of $OD_{620\ nm}$ in this case. If the microplate reader is not equipped with long wavelength measurement, this step can be omitted.
- The $OD_{450\text{-}620\text{-}N}$ value of calibration curve fitting points and samples should be calcualted by substracting the $OD_{450\text{-}620}$ of NCS, then take the average value of replicates.
- Perform a 4-parameter logistic regression model using the Calibration Standard concentration values and OD values to obtain the calibration curve equation. Substitute the average OD value of the sample into the equation to calculate the sample concentration, which should be multiplied by the dilution factor to obtain the actual sample concentration.
- The software for data analysis of the standard curve could be the one that comes with the microplate reader. If not, we recommend to use professional standard curve software such as Curve Expert, ELISA Calc, and so on.
- For samples with absorbance values above the Calibration standard ST1, a pilot study should be performed to determine an appropriate dilution before retesting. The Human IFN-γ concentration in the sample is calculated from the test value multiplied by its corresponding dilution factor. If the spiked samples are simultaneously set at this dilution level and the recovery rate should meet the requirements of the corresponding regulations.

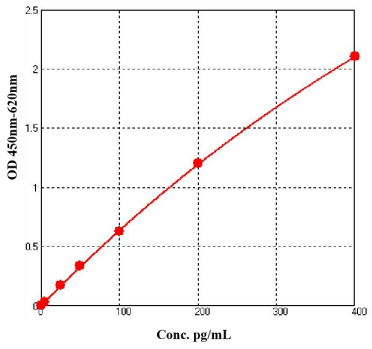
■ Limitations

- This product is intended for research use only but not for clinical use.
- The samples pH should be between 6.0 and 8.5. Beyond this range may cause abnormal results.

■ Assay Performance

- Linearity& Range: 5-400 pg/mL, $R^2 \geq 0.990$
- LLOQ: 5 pg/mL
- Typical calibration curve and data:

Calibration Standards (pg/mL)	Abs. At (450 nm-620 nm)	AVG
400	2.1379	2.1133
	2.1037	
	2.0982	
200	1.2186	1.2110
	1.2117	
	1.2028	
100	0.6453	0.6386
	0.6427	
	0.6277	
50	0.3519	0.3466
	0.3468	
	0.3411	
25	0.1834	0.1826
	0.1808	
	0.1836	
5	0.0441	0.0450
	0.0445	
	0.0463	
1	0.016	0.0162
	0.0165	
	0.016	
0	0.0097	0.0102
	0.0103	
	0.0105	



4-PL: $Y = \frac{A-D}{1+(\frac{X}{C})^B} + D$

A = 8.44302
B = -1.01023
C = 1192.73656
D = 0.00183
R^2 = 0.99994

- Specificity:

The assay recognizes natural and recombinant human IFN-γ.

No cross-reactivity with following factors.

a. Recombinant human: IL-2, IL-6, IL-7, IL-10, IL-12, IFN-β, IFN-α2b, IFN-γ R1, IFN-γ R2;

b. Other recombinants: Rat IFN-γ, Mouse IFN-γ.

- Calibration:

The assay is calibrated against recombinant human IFN-γ. The NIBSC/WHO Human IFN-γ International Standard 82/587 was evaluated in this kit.

NIBSC (82/587) approximate value (IU/mL) = 0.0065 × Human IFN-γ value (pg/mL)

■ Additional Information

- ✧ This kit is intended for use by qualified technicians only.
- ✧ Consumables, for example sterile disposable tips, tubes and reservoirs are only allowed for single use. It is recommended to wipe with 75% ethanol before and after each use. Follow the specified pipetting procedure carefully.
- ✧ Users should validate the assay before testing their samples.
- ✧ Dilution should be gentle and thorough to avoid excessive foaming.
- ✧ Stop Solution is 1M HCl. Avoid direct contact with eyes, skin, and clothing.
- ✧ Do not mix the kit reagents from different lot numbers.
- ✧ Use fresh sterile water or ultra-pure water, and ensure the water temperature does not exceed 37℃.
- ✧ Seal or cover the microplate during sample incubation to avoid liquid evaporation.
- ✧ Avoid drying the wells before substrate incubation.
- ✧ Store unused microtiter strips in a sealed bag with desiccant to prevent contamination.
- ✧ Centrifuge Anti-human IFN-γ : Biotinylated Conjugate (100×) and Streptavidin-HRP (100×) before use to avoid any loss of the reagent.
- ✧ To avoid pipetting errors, pipetting or sampling accurately for dilution of standards and samples, for example, a minimum volume of 5 μL is recommended.
- ✧ Human IFN-γ Calibration Standard Solutions, 1×Anti-Human IFN-γ:Biotinylated Conjugate and 1× Streptavidin-HRP are recommended for single use due to stability issue. Prepare freshly before each experiment.
- ✧ TMB Substrate should be colorless. If not, discard it and contact us for assistance.
- ✧ Plate reading should be completed within 30 minutes after termination.
- ✧ Avoid the samples containing sodium azide (NaN_3), which will deactivate the HRP and lead to the underestimation of Human IFN-γ levels.

■ Troubleshooting

Problem	Possible Cause	Solution
High background signal (OD)	Cross-contamination of reagents, including ultra-pure water	Freshly prepared prior to experiment.
	Cross-contamination of equipment, including pipettes and centrifuge	Clean the equipment with 75% ethanol before experiment.
	Environment contamination	Separate the working bench to avoid contamination.
	Insufficient washing	Increase the wash buffer volume or wash times, and remove any remaining liquid before proceeding to the next step.
Abnormal values	Improper washing	Swiftly and completely shake off any excess liquid, and avoid reusing paper towels to minimize contamination.
	Improper sampling	Add the samples to the bottom of the wells using pipettes, and avoid splashing to the neighboring wells.
	Plate sealing	Promptly cover the plate with the sealing film and remove it carefully to prevent splashing.

If you have any other questions, please contact us for technical support.

■ References

- ICH. M10 Bioanalytical Method Validation And Study Sample Analysis
- FDA. Bioanalytical Method Validation Guidance for Industry

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Support & Contact

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