Human IFN-gamma Precoated ELISA Kit

Catalog Number: EXHK027

For Research Use Only. Not Intended for Diagnostic or Therapeutic Use.

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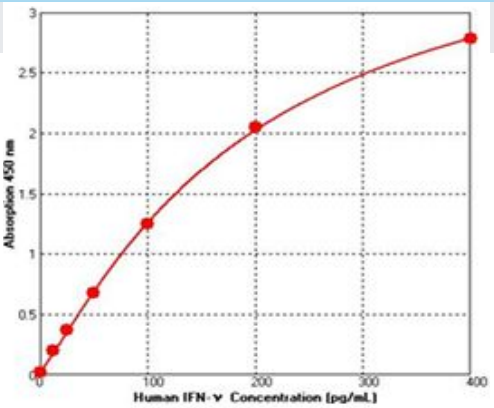
Product Description

The Human IFN-gamma Precoated ELISA Kit is used for the *in vitro* quantitative detection of human IFN-gamma in human serum, plasma, buffer or cell culture medium. Natural and recombinant human IFN-gamma are captured by the immobilized antibody coated on the microplate and detected by the biotinylated anti-IFN-gamma antibody followed by streptavidin-HRP and the tetramethylbenzidine (TMB) substrate. Read this manual in its entirety before use.

- Assay time: 2 hours 50 minutes
- Detection range: 12.5-400 pg/mL
- Sensitivity: 5 pg/mL
- Precision: Intra-assay CV: ≤10%; Inter-assay CV: ≤15%
- Specificity: no cross-reactivity with human cytokines



Example Standard Curve

Limitations

- For Research Use Only. Not Intended for Diagnostic or Therapeutic Use.
- Variations in temperature, incubation time or technique while performing the assay can affect results.
- Variations in collecting, processing and storing samples may affect sample values.
- Do not substitute kit components with reagents from other sources or lots.

Materials Provided, Storage and Reconstitution

Store the kit at 2-8ºC for up to 12 months. Do not use past kit expiration date.

Reagents	Quantity	Reconstitution
Human IFN-gamma Standard, lyophilized	2 bottles	Follow instructions on the vial. **Use a new standard for each assay. Discard after use.**
Biotinylated Human IFN-gamma Antibody	2 vials	1:50 dilution with Dilution Buffer R (1X)
Streptavidin-HRP	2 vials	1:100 dilution with Dilution Buffer R (1X)
Dilution Buffer R (1X)	3 bottles	Ready-to-use
Washing Buffer (50X)	1 bottle	1:50 dilution with distilled water
Color Reagent (TMB)	1 bottle	Ready-to-use
Stop Solution	1 bottle	Ready-to-use
Precoated Human IFN-gamma 96-well ELISA plate	1 plate (12 strips of 8 wells)	Ready-to-use
Plate Sealers	4 strips	Ready-to-use

Additional Required Supplies

- Microplate reader with 450 nm filter (with optional wavelength correction filter at 620 nm)
- Adjustable pipettes and pipette tips
- Distilled or deionized water
- Vortex mixer and magnetic stirrer.

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Precautions

- Store the kit according to the labels and instructions.
- Reagents should be at room temperature before use.
- Streptavidin-HRP and biotinylated antibody should be centrifuged briefly before use.
- Store unused plate strips in the sealed pouch.
- Avoid cross-contamination: change pipette tips between adding each standard dilution, between samples, and between reagents.
- Use separate reservoirs for individual reagents.
- Ensure reagents are mixed evenly by shaking or stirring. Avoid foaming of reagents.
- Add all reagents in the same order to the plate to ensure consistent reaction times for each step.
- Remove remaining wash buffer from wells completely after each wash step. Blot the inverted plate on clean filter paper or paper towels before adding the next reagent.
- Color Reagent (TMB) is sensitive to light and exposure to metals. Protect from light and avoid contact with metals. If unused Color Reagent is contaminated, it will turn blue before use and should be discarded appropriately.
- For each experiment, a standard curve must be generated.
- Results outside the linear range of the standard curve are not accurate. Dilute samples generating values above the highest standard with Dilution Buffer R (1X).

Preparation of Reagents

Human IFN-gamma Standard: Please refer to the product label for reconstitution instructions. Let stock solution in reconstituted vial stand for 5 minutes. Shake gently until completely dissolved. Dilute stock solution with Dilution Buffer R (1X) to 400 pg/mL, 200 pg/mL, 100 pg/mL, 50 pg/mL, 25 pg/mL and 12.5 pg/mL.

Biotinylated Human IFN-gamma Antibody: Dilute 1:50 with Dilution Buffer R (1X) in a clean tube according to the table below to prepare the 1X working solution. Mix well.

Number of Wells	Biotinylated Antibody (uL)	Dilution Buffer R (1X) (uL)
16	18	900
24	26	1300
32	34	1700
48	50	2500
96	100	5000

Streptavidin-HRP: Dilute 1:100 with Dilution Buffer R (1X) in a clean tube according to the table below to prepare the 1X working solution. Mix well.

Number of Wells	Streptavidin-HRP (uL)	Dilution Buffer R (1X) (uL)
16	17	1700
24	25	2500
32	34	3400
48	50	5000
96	100	10000

Wash Buffer (50X): Dilute 1:50 with distilled water to prepare 1X solution.

Protocol

1. Reagents and samples should be at room temperature before use.
2. Determine the required number of wells required for the samples, standard curve, and blank control to be tested in duplicate. Remove unneeded plate strips and store in sealed pouch.
3. Add 100 uL of standard and sample per well.
4. Add 100 uL of Dilution Buffer R (1X) to the blank control wells.
5. Add 50 uL of Biotinylated Human IFN-gamma Antibody (1X) to each well. Cover with the plate sealer and incubate for 2 hours at room temperature (18-25^{o}C).
6. Aspirate each well. Add 300 uL of Wash Buffer (1X) to each well. Let stand for 1minute. Repeat 3 times. Remove remaining wash buffer from wells completely after each wash step. Blot the inverted plate on clean filter paper or paper towels before add the next reagent.
7. Add 100 uL of streptavidin-HRP (1X) to each well. Cover with the plate sealer and incubate for 20 minutes at room temperature.
8. Repeat the wash steps, see Step 6.
9. Add 100 uL of Color Reagent to each well. Incubate for 10-30 minutes at room temperature. **Protect from light.**
10. Add 100 uL of Stop Solution to each well. A color change from blue to yellow should be observed. Gently tap the side of the plate to ensure thorough mixing.
11. Within 10 minutes, read the absorbance values using a microplate reader at 450 nm, with wavelength correction at 620 nm. The wavelength correction compensates for optical imperfections of the plate. Values measured at 450 nm without correction will be less accurate.

Data Analysis

Calculate the average optical density (O.D.) of the duplicate wells for the standards, samples and controls. Subtract the average blank control O.D value from all wells.

With suitable software, generate the standard curve using a four parameter (4-PL) curve-fit. The standard concentration is plotted on the x-axis and the mean O.D. value is plotted on the y-axis.

The software will report the calculated concentrations of the sample wells based on the standard curve. If the samples were diluted prior to adding to the wells, these calculated concentrations must be multiplied by the dilution factor.

Troubleshooting Guide

Problem	Possible Source	Suggested Action
Weak signal across entire plate	Incorrect washing method	Verify correct washing method and wash buffer
	Reagents have expired	Repeat assay with fresh reagents
	Streptavidin-HRP concentration is too low	Repeat assay according to recommended dilution of streptavidin-HRP
	Insufficient incubation time	Repeat assay according to recommended incubation time of each step
	Incorrect storage of reagents	Store reagents per recommended temperature, time and protect from light, as noted.
	Incorrect wavelength in microplate reader	Check filters in microplate reader

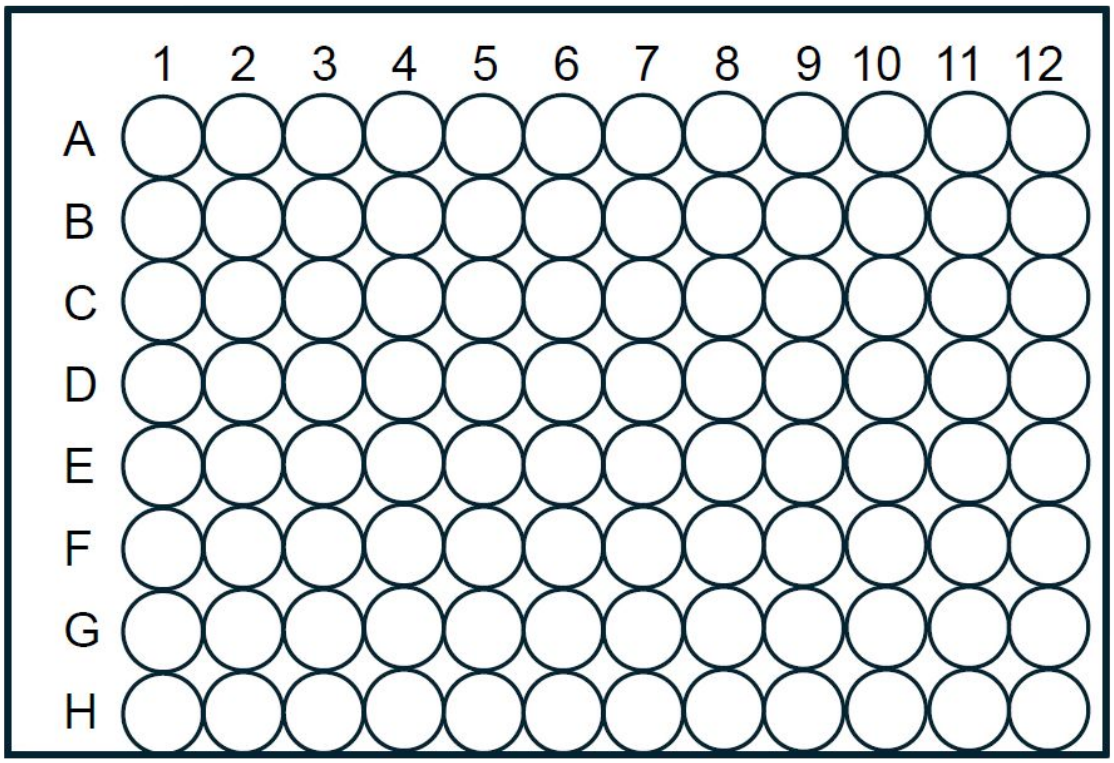
(Troubleshooting continued on next page)

Troubleshooting Guide (cont'd)

Problem	Possible Source	Suggested Action
Poor duplicates	Incorrect washing method	Verify correct washing method and wash buffer
	Plates are not clean	Wipe the bottom of the plate before reading the plate
	Incorrect wavelength in microplate reader	Check filters in microplate reader
	Reagents from different lots or sources were substituted in the kit	Only use reagents supplied in the kit
	Cross-contamination in the well	Use new pipette tips between samples, standards, and reagents
	Plate sealer reused or omitted	Use fresh plate sealer for each incubation
No signal (positive control or standard curve does not develop color)	Contamination of streptavidin-HRP with sodium azide	Use fresh reagents
	Protocol steps omitted or incorrect order	Review protocol and repeat with correct addition of reagents
	Reagents have expired	Repeat assay with fresh reagents
High background	Incorrect washing method	Verify correct washing method and wash buffer
	Color Reagent is contaminated or exposed to light	Verify Color Reagent is colorless before use and is stored properly
	Reagents have expired	Repeat assay with fresh reagents
	Incorrect reagent dilution, *e.g.* streptavidin-HRP or biotinylated antibody concentration too high	Repeat assay according to recommended dilution
	Contamination of wells with standards or positive samples	Repeat assay and avoid cross-contamination of wells
	Incubation time too long or temperature is too high	Repeat assay according recommended incubation time and temperature

Plate Layout

This template is provided to record layout of standards and samples.



Protocol At-A-Glance

Add 100 uL sample, standard and blank (diluent only) to each well

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Add 50uL diluted biotinylated antibody to each well

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Incubate for 2 hours at room temperature and wash 3 times

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Add 100 uL diluted streptavidin-HRP to each well

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Incubate for 20 minutes at room temperature and wash 3 times

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Add 100 uL of ready-to-use TMB to each well and incubate for 10-30 minutes (protect from light)

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Add 100 uL of Stop Solution to each well

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Read the plate at 450 nm (620 nm correction) within 10 minutes