Basic IFN gamma Human ELISA Kit

Enzyme-linked immunosorbent assay for quantitative detection of human IFN γ

Catalog Numbers ECH001 (96 tests)

Pub. No. MAN1000044 Rev. A00 (30)

WARNING! Read the Safety Data Sheets (SDSs) and follow the handling instructions. Wear appropriate protective eyewear, clothing, and gloves. Safety Data Sheets (SDSs) are available from thermofisher.com/support.

Product description

The Basic IFN gamma Human ELISA Kit is an enzyme-linked immunosorbent assay for the quantitative detection of human IFN γ.

Interferon gamma (IFN γ), also known as Type II interferon, is a macrophage activation factor and immune interferon primarily produced by T-lymphocytes and natural killer cells. IFN γ production is triggered by antigens, mitogens, Staphylococcus enterotoxin B, phytohemagglutinin, and other cytokines. IFN γ is a dimeric protein composed of two 146 amino acid subunits, acts as a glycoprotein homodimer with an approximate molecular weight of 45 kDa. On SDS-PAGE, IFN γ appears as a combination of bands at 25 and 20, and a minor band at 15.5 kDa due to differential glycosylation.

The biological activity of the IFN γ homodimer is highly species-specific, with no cross-reactivity observed between human and mouse. IFN γ exhibits diverse functions, including antiviral activity, tumor antiproliferative activity, induction of class I and II MHC, macrophage activation, and enhanced immunoglobulin secretion by B lymphocytes. It plays a crucial role in cytokine regulation and works synergistically with other cytokines. Activation of IFN γ occurs through binding to IFN γ receptor I and II, subsequently activating the JAK-STAT pathway. Notably, IFN γ does not share sequence homology with IFN alpha or IFN beta; however, human IFN γ shows approximately 40% sequence homology with mouse IFN γ. The expression of IFN γ is upregulated by IL2, FGF basic, EGF, and downregulated by vitamin D3 or DMN. Mutations in the IFN γ gene have been associated with aplastic anemia.

For literature updates, go to thermofisher.com.

Contents and storage

- Store kit reagents at 2–8°C.
- Immediately after use, return remaining reagents to cold storage (2–8°C).
- See the expiration date on the package.

Components	Amount
Coated Microwell Strips	1 pouch (12 strips with 8 wells each)
Biotin-Conjugate (100×)	70 µL
Streptavidin-HRP (100×)	150 µL
Human IFN γ Standard, lyophilized (200 ng/mL upon reconstitution)	2 vials
Sample Diluent	12 mL
Assay Buffer Concentrate 20×	5 mL
Wash Buffer Concentrate 20×	50 mL
Substrate Solution (Tetramethyl-benzidine)	15 mL
Stop Solution (1M Phosphoric acid)	15 mL
Adhesive Film	4

Required materials not supplied

- Distilled or deionized water
- Microtiter plate reader with software capable of measurement at 450 nm (620 nm as optional reference wavelength)
- Calibrated adjustable precision pipettes and glass or plastic tubes for diluting solutions
- Beakers, flasks, and cylinders for preparation of reagents
- Plate washer–automated or manual (squirt bottle, manifold dispenser, or equivalent)

Before you begin

- Equilibrate the buffer concentrates to room temperature, then dilute before use.
- If crystals have formed in the buffer concentrates, warm gently to dissolve the crystals.

Prepare Wash Buffer (1×)

1. Transfer the entire contents (50 mL) of the Wash Buffer Concentrate (20×) to a clean 1,000-mL graduated cylinder, then add 950 mL of glass-distilled or deionized water. Mix gently to avoid foaming.
2. Transfer to a clean wash bottle, then label as 1× Wash Buffer.
3. Store Wash Buffer (1×) at 2–25°C for up to 30 days.

Prepare Assay Buffer (1×)

1. Transfer the entire contents (5 mL) of the Assay Buffer Concentrate (20×) to a clean 100-mL graduated cylinder, then add 95 mL of distilled water. Mix gently to avoid foaming.
2. Label as 1× Assay Buffer.
3. Store Assay Buffer (1×) at 2–8°C for up to 30 days.

Prepare 1× Biotin-Conjugate

Make a 1:100 dilution of the concentrated Biotin-Conjugate solution with Assay Buffer (1×) in a clean plastic tube.

IMPORTANT! Prepare Biotin-Conjugate within 30 minutes of usage.

Dilute 0.06 mL of Biotin-Conjugate (100×) with 5.94 mL of Assay Buffer (1×), then mix thoroughly.

Prepare 1× Streptavidin-HRP

Make a 1:100 dilution of the concentrated Streptavidin-HRP Conjugate in a clean plastic tube.

IMPORTANT! Prepare 1× Streptavidin-HRP within 30 minutes of usage.

Dilute 0.12 mL of concentrated Streptavidin-HRP conjugate with 11.88 mL of Assay Buffer (1×), then mix thoroughly.

Prepare Human IFN γ Standard

Prepare fresh standard on each day of use as it cannot be stored.

1. Reconstitute Human IFN γ Standard by addition of distilled water. The reconstitution volume is stated on the label.

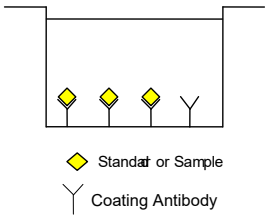
   Note: The concentration of the reconstituted standard is 200 ng/mL.

2. Before making dilutions, allow the standard to reconstitute for 10–30 minutes, then mix well.
3. Dilute the concentrated human IFN γ standard 1:1000 with Assay Buffer (1×) just prior to use in a clean plastic test tube according to the following dilution scheme:
   a. **Dilution 1**: Add 100 µL concentrated human IFN γ standard and 900 µL Assay Buffer (1×) (concentration of Dilution 1 = 20 ng/mL), then shake gently to mix.
   b. **Dilution 2**: Add 10 µL of Dilution 1 and 990 µL Assay Buffer (1×) (concentration of Dilution 2 = 200 pg/mL), then shake gently to mix.
4. Proceed to prepare standard dilutions on a microwell plate.

Perform ELISA protocol

Allow all components to reach room temperature before use. Mix all liquid reagents prior to use.

1 Bind antibody



1. Wash the microwell strips twice with approximately 400 µL of 1× Wash Buffer per well with thorough aspiration of microwell contents between washes. Allow the Wash Buffer to sit in the wells for about 10–15 seconds before aspiration. Do not scratch the surface of the microwells.

   After the last wash step, empty wells and tap microwell strips on absorbent pad or paper towel to remove excess Wash Buffer. Use the microwell strips immediately after washing. Alternatively, microwell strips can be placed upside down on a wet absorbent paper for not longer than 15 minutes. Do not allow wells to dry.

2. **Prepare Human IFN γ Standard dilutions on the microwell plate as follows:**
   a. Add 100 µL of Sample Diluent, in duplicate, to all standard wells.
   b. Add 100 µL of the diluted standard (see “Prepare Human IFN γ Standard” on page 2, concentration = 200 pg/mL), in duplicate, to wells A1 and A2.
   c. Mix the contents of wells A1 and A2 by repeated aspiration and ejection (concentration of standard 1, S1 = 100 pg/mL), then transfer 100 µL to wells B1 and B2, respectively (see Figure 1). Do not scratch the inner surface of the microwells.

1 *(continued)*

d. Repeat the above procedure 5 times, creating two rows of Human IFN γ Standard dilutions ranging from 100 pg/mL to 1.6 pg/mL. Discard 100 µL of the contents from the last microwell (G1/G2=S7) used.

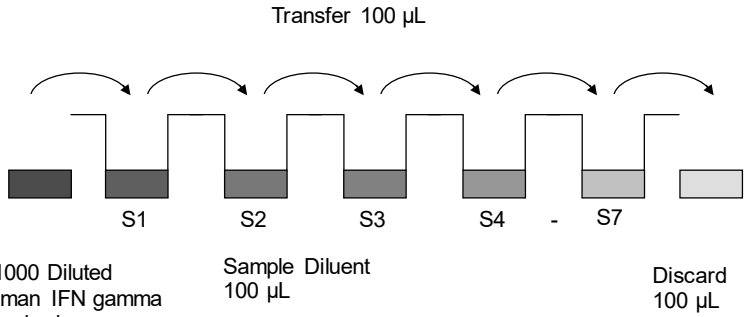
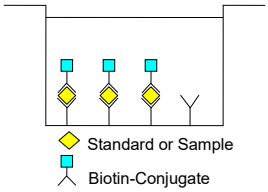


Figure 1 Standard dilutions on the microwell plate

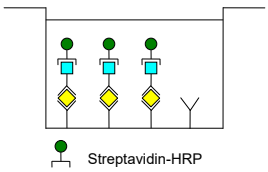
3. Add 100 µL of Sample Diluent in duplicate to the blank wells.
4. Add 50 µL of Sample Diluent to the sample wells.
5. Add 50 µL of each sample in duplicate to the sample wells.

2 Add 1× Biotin Conjugate



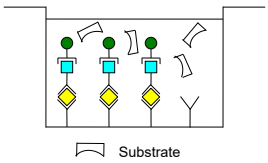
1. Add 50 µL of 1× Biotin-Conjugate (see “Prepare 1× Biotin-Conjugate” on page 2) to all wells.
2. Cover the plate with an adhesive film and incubate for 2 hours at room temperature (18–25°C), if available on a microplate shaker set at 400 rpm.
3. Prepare 1× Streptavidin-HRP as mentioned in “Prepare 1× Streptavidin-HRP” on page 2.
4. Remove adhesive film and empty wells. Thoroughly aspirate the solution and wash wells 3 times with 1× Wash Buffer. Allow the Wash Buffer to sit in the wells for 10–15 seconds for each wash before aspiration.

3 Add 1× Streptavidin-HRP Conjugate solution



1. Add 100 µL of 1× Streptavidin-HRP Conjugate (see “Prepare 1× Streptavidin-HRP” on page 2) to all wells, including the blanks wells.
2. Cover the plate with an adhesive film and incubate for 1 hour at room temperature (18–25°C), if available on a microplate shaker set at 400 rpm.
3. Remove adhesive film and empty wells. Thoroughly aspirate the solution and wash wells 3 times with 1× Wash Buffer.

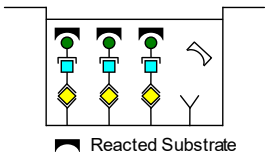
4 Add TMB Substrate Solution



1. Add 100 µL TMB Substrate Solution to all wells.
2. Incubate the microwell strips at room temperature (18–25°C) for about 10 minutes. Avoid direct exposure to intense light.

   Note: The color development on the plate should be monitored and the substrate reaction stopped (see next step) before positive wells are no longer properly recordable. Determination of the ideal time period for color development has to be done individually for each assay.

5 Add Stop Solution



It is recommended to add the stop solution when the highest standard develops a dark blue color.

Add 100 µL Stop Solution to each well. Tap the side of the plate to mix. The solution in the wells changes from blue to yellow.

IMPORTANT! It is important that the Stop Solution is spread quickly and uniformly throughout the microwells to completely inactivate the enzyme.

Calculation of results

Read the absorbance on a spectrophotometer using 450 nm as the primary wavelength (optionally 620 nm as the reference wavelength; 610 nm to 650 nm is acceptable as well). Blank the plate reader according to the manufacturer's instructions by using the blank wells. Determine the absorbance of both the samples and the controls.

IMPORTANT! Results must be read immediately after the Stop Solution is added or within one hour if the microwell strips are stored at 2–8°C in the dark.

Note: If the instructions in this protocol have been followed and samples have been diluted 1:2 (50 µL of sample + 50 µL of Sample Diluent), multiply each concentration by 2.

A representative standard curve is shown in Figure 2.

Note: Do not use this standard curve to derive test results. Each laboratory must prepare a standard curve for each group of microwell strips assayed.

Human IFN γ was diluted in serial 2-fold steps in Sample Diluent.

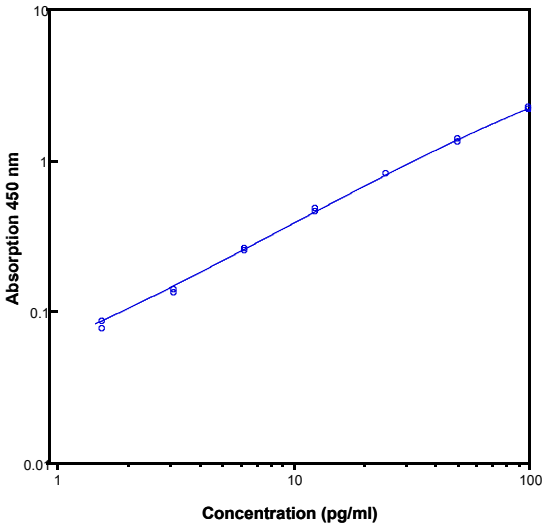


Figure 2 Representative standard curve for human IFN γ ELISA

Table 1 Typical data using the human IFN γ ELISA

Standard	Human IFN γ Concentration (pg/mL)	O.D. at 450 nm	Mean O.D. at 450 nm	C.V. (%)
1	100.00	2.210 2.143	2.177	2.2
2	50.0	1.307 1.376	1.342	3.6
3	25.0	0.802 0.805	0.804	0.3
4	12.5	0.450 0.475	0.463	3.8
5	6.3	0.257 0.250	0.254	2.0
6	3.1	0.139 0.132	0.136	3.7
7	1.6	0.085 0.077	0.081	7.0
Blank	0	0.027 0.027	0.027	0

The OD values of the standard curve may vary according to the conditions of assay performance (for example, operator, pipetting technique, washing technique, or temperature effects).

Performance characteristics

Sensitivity

The limit of detection of human IFN γ defined as the analyte concentration resulting in an absorbance significantly higher than that of the dilution medium (mean plus 2 standard deviations) was determined to be 0.99 pg/mL (mean of 6 independent assays).

Specificity

The interference of circulating factors of the immune system was evaluated by spiking these proteins at physiologically relevant concentrations into a human IFN γ positive serum. No cross-reactivity was detected.

Expected values

Panels of 40 serum as well as EDTA, citrate, and heparin plasma samples from randomly selected apparently healthy donors (males and females) were tested for human IFN γ. Elevated human IFN γ levels depend on the type of immunological disorder. The levels measured may vary with the sample collection used.

Table 2 Expected values

Sample matrix	Number of samples valuated	Range (pg/mL)	Detectable (%)	Mean of detectable (pg/mL)
Serum	40	nd[1]–188.9	10.0	55.7
Plasma (EDTA)	40	nd[1]–9.1	7.5	6.0
Plasma (citrate)	40	nd[1]–4.0	2.5	–
Plasma (heparin)	40	nd[1]–4.3	2.5	–

[1] nd = nondetectable, samples measured below the lowest standard point are considered to be nondetectable.

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  Note: For SDSs for reagents and chemicals from other manufacturers, contact the manufacturer.

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For descriptions of symbols on product labels or product documents, go to thermofisher.com/symbols-definition.

Revision history: Pub. No. MAN1000044 A00 (30)

Revision	Date	Description
A00 (30)	25 April 2024	New document for Basic IFN gamma Human ELISA Kit.