

ab46048 – IFN gamma High Sensitivity Human ELISA Kit

Instructions for Use

For the quantitative measurement of interferon gamma (IFN gamma) in Human sera, plasmas, buffered solutions, and cell culture media.

This product is for research use only and is not intended for diagnostic use.

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1. BACKGROUND

Abcam's IFN gamma Human High Sensitivity *in vitro* ELISA (Enzyme-Linked Immunosorbent Assay) kit is designed for the quantitative measurement of IFN gamma in Human sera, plasmas, buffered solutions and cell culture media.

A monoclonal antibody specific for IFN gamma has been coated onto the wells of the microtiter strips provided. Samples, including standards of known IFN gamma concentrations, control specimens or unknowns are pipetted into these wells. During the first incubation, the standards or samples and a biotinylated monoclonal antibody specific for IFN gamma are simultaneously incubated. After washing, the enzyme Streptavidin-HRP, that binds the biotinylated antibody is added, incubated and washed. A TMB substrate solution is added which acts on the bound enzyme to induce a colored reaction product. The intensity of this colored product is directly proportional to the concentration of IFN gamma present in the samples.

This kit will recognize both endogenous and recombinant Human IFN gamma.

2. ASSAY SUMMARY

Primary capture antibody



Sample



Primary detector antibody



Conjugated secondary antibody



Substrate **Colored product**



Remove appropriate number of antibody coated well strips. Equilibrate all reagents to room temperature. Prepare all the reagents, samples, and standards as instructed.

Add standard or sample to each well used.

Add prepared Biotinylated labeled detector antibody. Incubate at room temperature.

Aspirate and wash each well. Add prepared Streptavidin-HRP mix to each well. Incubate at room temperature.

Aspirate and wash each well. Add the TMB Solution to each well until color develops and then add the Stop Solution. Immediately begin recording the color development.

3. PRECAUTIONS

Please read these instructions carefully prior to beginning the assay.

All kit components have been formulated and quality control tested to function successfully as a kit. Modifications to the kit components or procedures may result in loss of performance.

4. STORAGE AND STABILITY

Store kit at +2-8°C immediately upon receipt.

Refer to list of materials supplied for storage conditions of individual components. Observe the storage conditions for individual prepared components in section 9. Reagent Preparation.

5. MATERIALS SUPPLIED

Item	Quantity		Storage Condition (Before Preparation)
	1 x 96 tests	2 x 96 tests	
IFN gamma Microplate (12 x 8 well strips)	96 wells	2 x 96 wells	+2-8°C
IFN gamma Standard (Lyophilized)	2 vials	4 vials	+2-8°C
10X Standard Diluent Buffer	15 mL	25 mL	+2-8°C
Biotinylated anti-IFN gamma	400 µL	2 x 400 µL	+2-8°C
Biotinylated Antibody Diluent	7.5 mL	13 mL	+2-8°C
Streptavidin-HRP	2 x 5 µL	4 x 5 µL	+2-8°C
HRP Diluent	23 mL	2 x 23 mL	+2-8°C
Amplifier*	200 µL	2 x 200 µL	+2-8°C
Amplification Diluent	15 mL	25 mL	+2-8°C
200X Wash Buffer	10 mL	2 x 10 mL	+2-8°C
Chromogen TMB Substrate Solution	11 mL	24 mL	+2-8°C
Stop Reagent	11 mL	2 x 11 mL	+2-8°C
Plastic plate cover	2 units	4 units	+2-8°C

*Reagent contains ethyl alcohol

Note: This ELISA kit will soon contain the “Easy View” colored reagents. The Standard diluent buffer will now be red, and the Streptavidin-HRP Diluent will be green. Please note that while stock lasts you may still receive colorless diluents. This change does not impact the results provided by the kit or the assay procedure.

6. MATERIALS REQUIRED, NOT SUPPLIED

These materials are not included in the kit, but will be required to successfully utilize this assay:

- Microplate reader capable of measuring absorbance at 450 nm.
- Precision pipettes to deliver 2 μ L to 1 mL volumes.
- Adjustable 1-25 mL pipettes for reagent preparation.
- 100 mL and 1 liter graduated cylinders.
- Absorbent paper.
- Distilled or deionized water.
- Tubes to prepare standard or sample dilutions.
- Log-log graph paper or computer and software for ELISA data analysis.

7. LIMITATIONS

- Do not mix or substitute reagents or materials from other kit lots or vendors. Kits are QC tested as a set of components and performance cannot be guaranteed if utilized separately or substituted.
- Since exact conditions may vary from assay to assay, a standard curve must be established for every assay performed.
- Bacterial or fungal contamination of either samples or reagents or cross-contamination between reagents may cause erroneous results.
- Disposable pipette tips, flasks or glassware are preferred, reusable glassware must be washed and thoroughly rinsed of all detergents before use.
- Improper or insufficient washing at any stage of the procedure will result in either false positive or false negative results. Completely empty wells before dispensing fresh 1X Wash Buffer. Do not allow wells to sit uncovered or dry for extended periods.

8. TECHNICAL HINTS

- Kit components should be stored as indicated. All the reagents should be equilibrated to room temperature before use. Reconstituted standards should be discarded after use.
- Once the desired number of strips has been removed, immediately reseal the bag to protect the remaining strips from degradation.
- Use a clean disposable plastic pipette tip for each reagent, standard, or specimen addition in order to avoid cross-contamination; for the dispensing of the Stop Solution and substrate solution, avoid pipettes with metal parts.
- Thoroughly mix the reagents and samples before use by agitation or swirling.
- All residual washing liquid must be drained from the wells by efficient aspiration or by decantation followed by tapping the plate forcefully on absorbent paper. Never insert absorbent paper directly into the wells.
- The TMB solution is light sensitive. Avoid prolonged exposure to light. Also, avoid contact of the TMB solution with metal to prevent color development. Warning TMB is toxic avoid direct contact with hands. Dispose off properly.
- If a dark blue color develops within a few minutes after preparation, this indicates that the TMB solution has been contaminated and must be discarded. Read absorbances within 1 hour after completion of the assay.
- When pipetting reagents, maintain a consistent order of addition from well-to-well. This will ensure equal incubation times for all wells.
- Dispense the TMB solution within 15 minutes following the washing of the microtiter plate.

- **This kit is sold based on number of tests. A ‘test’ simply refers to a single assay well. The number of wells that contain sample, control or standard will vary by product. Review the protocol completely to confirm this kit meets your requirements. Please contact our Technical Support staff with any questions.**

9. REAGENT PREPARATION

Equilibrate all reagents and samples to room temperature (18-25°C) prior to use.

9.1 1X Standard Diluent Buffer

Dilute the 10X Standard Diluent Buffer 10-fold in distilled water before use.

9.2 1X Wash Buffer

Dilute the 200X Wash Buffer Concentrate 200-fold in distilled water before use. Mix gently to avoid foaming. The 1X Wash Buffer can be prepared as needed according to the following table:

Number of well strips used	Volume of 200X Wash Buffer Concentrate (mL)	Volume of distilled water (mL)
1-6	5	995
1-12	10	1,990

9.3 1X Amplifier

It is recommended this reagent is prepared immediately before use. Dilute the Amplifier with the Amplification diluent in an appropriate clean glass vial using volumes appropriate to the number of required wells. Please see example volumes below:

Number of well strips used	Amplifier (µL)	Amplification Diluent (mL)
2	20	1.980
4	40	3.960
6	60	5.940
12	120	11.880

9.4 1X Biotinylated anti-IFN gamma

Prepare the 1X Biotinylated anti-IFN gamma immediately prior to use. According to the table below, dilute the Biotinylated anti-IFN gamma with the Biotinylated Antibody Diluent based on the number of wells being used in the assay procedure:

Number of well strips used	Volume of Biotinylated anti-IFN gamma (μL)	Volume of Biotinylated Antibody Diluent (μL)
2	40	1,060
3	60	1,590
4	80	2,120
6	120	3,180
12	240	6,360

9.5 1X Streptavidin-HRP Solution

Add 500 μL of HRP-Diluent to the Streptavidin-HRP vial prior to use to create a Streptavidin-HRP Concentrate. Do not keep this solution for further experiments.

Subsequently, prior to use in the assay procedure, prepare the 1X Streptavidin-HRP Solution by further diluting the Streptavidin-HRP Concentrate with HRP-Diluent. Use the table below to determine the volumes of each solution required to prepare the final 1X Streptavidin-HRP Solution:

Streptavidin-HRP Solution 1

Number of well strips used	Volume of Streptavidin-HRP (μL)	Volume of HRP-Diluent (mL)
2	20	1.980
4	40	3.960
6	60	5.940
12	120	11.880

Streptavidin-HRP Solution 2

Number of well strips used	Volume of Streptavidin-HRP (μL)	Volume of HRP-Diluent (mL)
2	40	1.960
4	80	3.920
6	120	5.880
12	240	11.760

10. STANDARD PREPARATION

Prepare serially diluted standards immediately prior to use. Always prepare a fresh set of standards for every use.

- 10.1 Prepare a 25 pg/mL **Standard #1** by reconstituting with the volume indicated on the vial using 1X Standard Diluent Buffer.
- 10.2 Label tubes #2-6 and add 100 μL of appropriate diluent into each tube.
- 10.3 Prepare **Standard #2** by adding 100 μL of Standard #1 to tube #2 and mix thoroughly.
- 10.4 Prepare **Standard #3** by adding 100 μL of Standard #2 to tube #3 and mix thoroughly.
- 10.5 Using the table below as a guide, prepare further serial dilutions.
- 10.6 1X Standard Diluent Buffer serves as the zero standard (0 pg/mL).

ASSAY PREPARATION

Standard Dilution Preparation Table

Standard #	Volume to Dilute (μL)	Diluent (μL)	Total Volume (μL)	Starting Conc. (pg/mL)	Final Conc. (pg/mL)
1	-	-	-	25	25
2	100	100	200	25	12.5
3	100	100	200	12.5	6.25
4	100	100	200	6.25	3.125
5	100	100	200	3.125	1.56
6	100	100	200	1.56	0.78



11. SAMPLE PREPARATION AND STORAGE

- **Preparation of Plasma Samples**

Collect plasma using citrate, EDTA or heparin. Centrifuge samples at 1,000 x *g* for 30 minutes. Dilute samples into 1X Standard Diluent Buffer and assay. Store plasma samples at -20°C or below for up to 3 months. Avoid repeated freeze-thaw cycles.

- **Preparation of Serum Samples**

Samples should be collected into a serum separator tube. After clot formation, centrifuge samples at 1,000 x *g* for 10 minutes and collect serum. Dilute samples into 1X Standard Diluent Buffer and assay. Store serum at -20°C or below. Avoid repeated freeze-thaw cycles.

- **Preparation of Cell culture Supernatants**

Centrifuge cell culture media at 1,000 x *g* for 10 minutes to remove debris. Collect supernatants and assay. Store samples at -20°C or below. Avoid repeated freeze-thaw cycles.

Normal sera and plasmas may be applied undiluted. Nevertheless, sera or plasmas from patients with various pathologies may be applied undiluted and diluted (to prevent too high concentrations). Please note that certain sera/plasma may induce false positive (for example, in reason to presence of anti-mouse anti-IgG antibody). A simple sample dilution (1:2) allows eliminating interference. As IFN gamma concentrations may vary considerably in cell supernatant samples, it is not easy to recommend a dilution factor.

12. PLATE PREPARATION

- The 96 well plate strips included with this kit is supplied ready to use. It is not necessary to rinse the plate prior to adding reagents.
- Unused well strips should be returned to the plate packet and stored at 4°C.
- For statistical reasons, we recommend each sample should be assayed with a minimum of two replicates (duplicates).
- Well effects have not been observed with this assay. Contents of each well can be recorded on the template sheet included in the Resources section.

13. ASSAY PROCEDURE

- **Equilibrate all materials and prepared reagents to room temperature prior to use.**
 - **It is recommended to assay all standards, controls and samples in duplicate.**
- 13.1 Prior to use, mix all reagents thoroughly taking care not to create any foam within the vials.
 - 13.2 Determine the number of microplate strips required to test the desired number of samples, plus appropriate number of wells needed for controls and standards. Remove sufficient microplate strips from the pouch.
 - 13.3 Add 100 μL of each standard (see Section 10), including blank controls to the appropriate wells.
 - 13.4 Add 100 μL of sample to the appropriate wells.
 - 13.5 Cover with a plastic plate cover and incubate at room temperature (18 to 25°C) with slow shaking for 1 hour.
 - 13.6 Remove the cover and wash the plate as follows:
 - 13.6.1 Aspirate the liquid from each well.
 - 13.6.2 Add 300 μL of 1X Wash Buffer into each well
 - 13.6.3 Aspirate the liquid from each well.
 - 13.6.4 Repeat for a total of 3 washes.
 - 13.7 Add 50 μL of 1X Biotinylated anti-IFN gamma to all wells (see Section 9).
 - 13.8 Cover and incubate for 1 hour at room temperature (18-25°C).
 - 13.9 Repeat Step 13.6
 - 13.10 Add 100 μL of 1X Streptavidin-HRP 1 solution into all wells, including the blank wells. Re-cover and incubate at room temperature for 20 minutes.
 - 13.11 Wash as described in Step 13.6
 - 13.12 Add 100 μL of diluted Amplifier to all wells

ASSAY PROCEDURE

- 13.13 Cover with a plastic plate cover and incubate at room temperature (18-25°C) with slow shaking for 15min.
- 13.14 Repeat wash step 13.6
- 13.15 Add 100 µL of 1X Streptavidin-HRP 2 solution into all wells
- 13.16 Cover with a plastic plate cover and incubate at room temperature (18-25°C) with slow shaking for 20min.
- 13.17 Repeat wash step 13.6
- 13.18 Add 100 µL of Chromogen TMB substrate solution into each well and incubate in the dark for 10-20 minutes at room temperature. Avoid direct exposure to light by wrapping the plate in aluminum foil. *Note:* Incubation time of the substrate solution is usually determined by the microplate reader performances: many microplate readers record absorbance only up to 2.0 O.D. The O.D. values of the plate should be monitored and the substrate reaction stopped before positive wells are no longer accurately readable (maximum ~20 minutes).
- 13.19 Add 100 µL of Stop Reagent into each well. Results must be taken immediately after the addition of Stop Reagent, or within one hour, if the microplate is stored at 2-8°C in the dark.
- 13.20 Read absorbance of each well on a spectrophotometer using 450 nm as the primary wavelength and optionally 620 nm (610 nm to 650 nm is acceptable) as the reference wavelength.

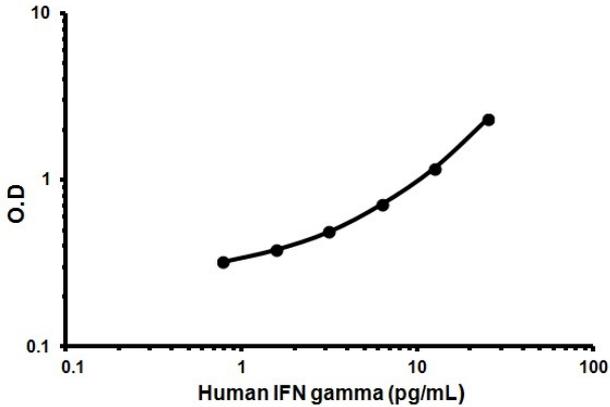
14. CALCULATIONS

Calculate the mean absorbance for each set of duplicate standards, controls, and samples, and subtract the average zero standard optical density. Plot the standard curve on log-log graph paper, with standard concentration on the x-axis and absorbance on the y-axis. Draw the best-fit straight line through the standard points.

For samples that have been diluted, the concentration read from the standard curve has to be multiplied by the dilution factor to determine the actual concentration of the target protein present.

15. TYPICAL DATA

TYPICAL STANDARD CURVE - Data provided for demonstration purposes only. A new standard curve must be generated for each assay performed.



Conc. (pg/mL)	O.D.
25	2.297
12.5	1.172
6.25	0.712
3.12	0.487
1.56	0.381
0.78	0.322
0	0.236

16. TYPICAL SAMPLE VALUES

EXPECTED VALUES -

16 sera and 16 plasmas from healthy individual donors were tested undiluted and diluted in duplicates. Mean concentration of IFN gamma in sera was 2.3 pg/mL (12 positives – range values of positive sera: 0.70 - 5.82 pg/mL) and in plasma is 1.89 pg/mL (13 positives - range values of positive plasmas: 0.89 - 5.08pg/mL).

SENSITIVITY -

The sensitivity, minimum detectable dose of Human IFN gamma using this Human IFN gamma ELISA kit was found to be <0.69 pg/mL. This was determined by adding 3 standard deviations to the mean OD obtained when the zero standard was assayed 40 times.

PRECISION -

	Intra- Assay	Inter- Assay
n=	6	6
CV (%)	3.9	8.6

DILUTION PARALLELISM -

Two Human serum pools, one Human plasma pool and one cell culture medium samples with various concentrations of IFN gamma were serially diluted in 1X Standard Diluent Buffer. Linearity was evaluated on 4 dilutions. The linear regression of samples versus the expected concentrations yielded a quote slope of 0.994.

SPIKE RECOVERY -

The spike recovery was evaluated by spiking two levels of IFN gamma into three Human serum pools, two plasma pools and two culture media. Recovery was evaluated with one test. Mean recoveries were 92% in sera (range values: 84-98%), 72% in plasma (Range values: 61-81%) and 108% in cell culture media (range values: 88-117%).

17. ASSAY SPECIFICITY

Ten specificities were tested with concentrations higher than IFN gamma curve concentrations. No cross reaction was observed for concentrations ranging from 2500 to 78.12 pg/mL for IL-1 α , IL-2, IL-8, IL-12p40, TNF- α , CD95 Fas, TRAIL, ICAM-1, Gp130 and GM-CSF.

18. TROUBLESHOOTING

Problem	Cause	Solution
Poor standard curve	Inaccurate pipetting	Check pipettes
	Improper standards dilution	Prior to opening, briefly spin the stock standard tube and dissolve the powder thoroughly by gentle mixing
Low Signal	Incubation times too brief	Ensure sufficient incubation times; change to overnight standard/sample incubation
	Inadequate reagent volumes or improper dilution	Check pipettes and ensure correct preparation
Large CV	Plate is insufficiently washed	Review manual for proper wash technique. If using a plate washer, check all ports for obstructions
	Contaminated wash buffer	Prepare fresh wash buffer
Low sensitivity	Improper storage of the ELISA kit	Store the reconstituted protein at -80°C, all other assay components 4°C. Keep substrate solution protected from light.

19. NOTES

Technical Support

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