



**LEGEND MAX<sup>™</sup>**  
ELISA Kit



**Human IFN- $\gamma$**

Cat. No. 430107

ELISA Kit for Accurate Quantitation of Human IFN- $\gamma$  from  
Cell Culture Supernatant, Serum, Plasma and Other  
Biological Fluids

BioLegend, Inc.  
[biolegend.com](http://biolegend.com)

***It is highly recommended that this manual be read in its entirety before using this product. Do not use this kit beyond the expiration date.***

*For Research Purposes Only. Not for use in diagnostic or therapeutic procedures. Purchase does not include or carry the right to resell or transfer this product either as a stand-alone product or as a component of another product. Any use of this product other than the permitted use without the express written authorization of BioLegend is strictly prohibited.*



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# LEGEND MAX™ Human IFN- $\gamma$ ELISA Kit

## Introduction:

Human IFN- $\gamma$  (Immune interferon, Type II interferon, T cell interferon, Macrophage-activating factor (MAF), IFN- $\gamma$ , IFN-gamma, etc.) is a potent multifunctional cytokine which is secreted primarily by activated NK cells and T cells. Originally characterized based on its anti-viral activities, IFN- $\gamma$  also exerts anti-proliferative, immunoregulatory, and proinflammatory activities. IFN- $\gamma$  can upregulate MHC class I and II antigen expression by antigen-presenting cells. Natural IFN- $\gamma$  is a dimeric protein with subunits of 143 amino acids. IFN- $\gamma$  is synthesized as a precursor protein of 166 amino acids including a secretory signal sequence of 23 amino acids. The protein is glycosylated.

The BioLegend LEGEND MAX™ Human IFN- $\gamma$  ELISA Kit is a Sandwich Enzyme-Linked Immunosorbent Assay (ELISA) with a 96-well strip plate that is pre-coated with a mouse monoclonal anti-human IFN- $\gamma$  antibody. The detection antibody is a biotinylated mouse monoclonal anti-human IFN- $\gamma$  antibody. This kit is specifically designed for the accurate quantitation of human IFN- $\gamma$  from cell culture supernatant, serum, plasma, and other biological fluids. This kit is analytically validated with ready-to-use reagents.

## Materials Provided:

Description	Quantity	Volume (per bottle)	Part #
Anti-human IFN- $\gamma$ Pre-coated 96-well Strip Microplate	1 plate		750002751
Human IFN- $\gamma$ Detection Antibody	1 bottle	12 mL	78318
Human IFN- $\gamma$ Standard	1 vial	lyophilized	79103
Avidin-HRP A	1 bottle	12 mL	79131
Assay Buffer A	1 bottle	25 mL	78232
Wash Buffer (20X)	1 bottle	50 mL	78233
Substrate Solution F	1 bottle	12 mL	79132
Stop Solution	1 bottle	12 mL	79133
Plate Sealers	1 pack		78101

**Materials to be Provided by the End-User:**

- Microplate reader able to measure absorbance at 450 nm
- Adjustable pipettes to measure volumes ranging from 1 μL to 1,000 μL
- Deionized water
- Wash bottle or automated microplate washer
- Log-Log graph paper or software for data analysis
- Tubes to prepare standard dilutions
- Timer
- Plate Shaker
- Polypropylene vials

**Storage Information:**

Store unopened kit components between 2°C and 8°C. Do not use this kit beyond its expiration date.

<b>Opened or Reconstituted Components</b>	
Microplate wells	If all microplate strips are not used, remove the excess strips by pressing up from underneath each strip. Place excess strips back in the foil pouch with the included desiccant pack and reseal. Store between 2°C and 8°C for up to one month.
Standard	The remaining reconstituted standard stock solution can be aliquoted into polypropylene vials and stored at -70°C for up to one month. Avoid repeated freeze-thaw cycles.
Detection Antibody	Store opened reagents between 2°C and 8°C and use within one month.
Avidin-HRP A	
Assay Buffer A	
Wash Buffer (20X)	
Substrate Solution F	
Stop Solution	

**Health Hazard Warnings:**

1. Reagents that contain preservatives may be harmful if ingested, inhaled or absorbed through the skin. Refer to the MSDS online at BioLegend’s website for details ([www.biolegend.com/msds](http://www.biolegend.com/msds)).
2. Substrate Solution F is harmful if inhaled or ingested. Avoid skin, eye and clothing contact.

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3. To reduce the likelihood of blood-borne transmission of infectious agents, handle all serum, plasma and other biological fluids in accordance with NCCLS regulations.
4. Stop Solution contains strong acid. *Wear eye, hand, and face protection.*
5. Before disposing of the plate, rinse it with an excess amount of tap water.

### Specimen Collection and Handling:

Specimens should be clear and non-hemolyzed. If possible, unknown samples should be run at a number of dilutions to determine the optimal dilution factor that will ensure accurate quantitation.

Cell Culture Supernatant: If necessary, centrifuge all samples to remove debris prior to analysis. It is recommended that samples be stored at  $< -70^{\circ}\text{C}$ . Avoid repeated freeze-thaw cycles.

Serum: Use a serum separator tube and allow clotting for at least 30 minutes, then centrifuge for 10 minutes at  $1,000 \times g$ . Remove serum layer and assay immediately or store serum samples at  $< -70^{\circ}\text{C}$ . Avoid repeated freeze-thaw cycles.

Plasma: Collect blood samples in citrate, heparin or EDTA containing tubes. Centrifuge for 10 minutes at  $1,000 \times g$  within 30 minutes of collection. Assay immediately or store plasma samples at  $< -70^{\circ}\text{C}$ . Avoid repeated freeze-thaw cycles.

### Reagent and Sample Preparation:

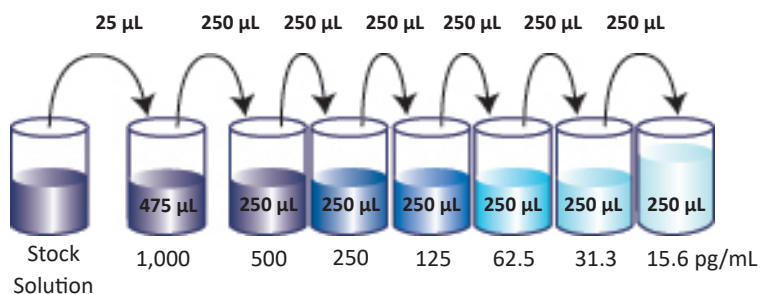
Note: All reagents should be diluted immediately prior to use.

1. Dilute the 20X Wash Buffer to 1X with deionized water. For example, make 1 liter of 1X Wash Buffer by adding 50 mL of 20X Wash Buffer to 950 mL of deionized water. If crystals have formed in the 20X Wash Buffer, bring to room temperature and vortex until dissolved.
2. Reconstitute the lyophilized Human IFN- $\gamma$  Standard by adding the volume of Assay Buffer A to make 20 ng/mL standard stock solution (refer to LEGEND MAX Kit Lot-Specific Certificate of Analysis/LEGEND MAX Kit Protocol). Allow the reconstituted standard to sit at room temperature for 15-20 minutes, then briefly vortex to mix completely.
3. In general, samples are analyzed without dilutions. However, if dilutions are required, use Assay Buffer A as the sample diluent.

## Assay Procedure:

Note: Do not mix reagents from different kits or lots. Reagents and/or antibodies from different manufacturers should not be used with this kit.

1. Bring all reagents to room temperature prior to use. It is strongly recommended that all standards and samples be run in duplicate or triplicate. A standard curve is required for each assay.
2. If all microplate strips will not be used, remove the excess strips by pressing up from underneath each strip. Place excess strips back in the foil pouch with the included desiccant pack and reseal.
3. Prepare 500  $\mu\text{L}$  of the 1,000 pg/mL top standard by diluting 25  $\mu\text{L}$  of the standard stock solution in 475  $\mu\text{L}$  of Assay Buffer A. Perform six two-fold serial dilutions of the 1,000 pg/mL top standard in separate tubes using Assay Buffer A as the diluent. Thus, the human IFN- $\gamma$  standard concentrations in the tubes are 1,000 pg/mL, 500 pg/mL, 250 pg/mL, 125 pg/mL, 62.5 pg/mL, 31.3 pg/mL and 15.6 pg/mL, respectively. Assay Buffer A serves as the zero standard (0 pg/mL).



4. Wash the plate 4 times with at least 300  $\mu\text{L}$  of 1X Wash Buffer per well and blot any residual buffer by firmly tapping the plate upside down on absorbent paper. All subsequent washes should be performed similarly.
5. Add 50  $\mu\text{L}$  of Assay Buffer A to each well that will contain either standard dilutions or samples.
6. Add 50  $\mu\text{L}$  of standard dilutions or samples to the appropriate wells
7. Seal the plate with a Plate Sealer included in the kit and incubate the plate at room temperature for 2 hours while shaking at 200 rpm.
8. Discard the contents of the plate into a sink, then wash the plate 4 times with 1X Wash Buffer as in step 4.

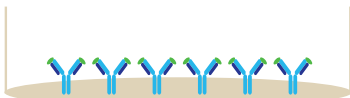
## LEGEND MAX™ Human IFN- $\gamma$ ELISA Kit

9. Add 100  $\mu$ L of Human IFN- $\gamma$  Detection Antibody solution to each well, seal the plate and incubate at room temperature for 1 hour while shaking.
10. Discard the contents of the plate into a sink, then wash the plate 4 times with 1X Wash Buffer as in step 4.
11. Add 100  $\mu$ L of Avidin-HRP A solution to each well, seal the plate and incubate at room temperature for 30 minutes while shaking.
12. Discard the contents of the plate into a sink, then wash the plate 5 times with 1X Wash Buffer as in step 4. For this final wash, soak wells in 1X Wash Buffer for 30 seconds to 1 minute for each wash. This will help minimize background.
13. Add 100  $\mu$ L of Substrate Solution F to each well and incubate for 15 minutes in the dark. Wells containing human IFN- $\gamma$  should turn blue in color with an intensity proportional to its concentration. It is not necessary to seal the plate during this step.
14. Stop the reaction by adding 100  $\mu$ L of Stop Solution to each well. The solution color should change from blue to yellow.
15. Read absorbance at 450 nm within 30 minutes. If the reader is capable of reading at 570 nm, the absorbance at 570 nm can be subtracted from the absorbance at 450 nm.

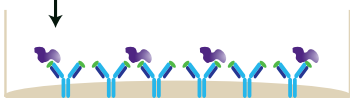


## Assay Procedure Summary

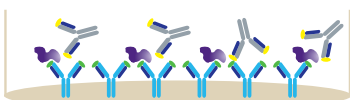
1. Wash 4 times  
Add 50  $\mu$ L Assay Buffer A



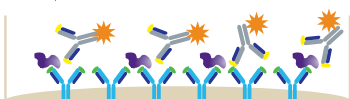
The diagram shows a rectangular well containing a layer of immobilized antibodies. Each antibody is represented as a blue Y-shaped structure with a green tip. They are arranged in a single layer on a light brown surface.
2. Add 50  $\mu$ L diluted standards or samples  
Incubate 2 hr., RT, shaking



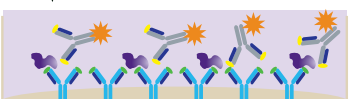
The diagram shows the same well as in step 1, but now with purple Y-shaped structures (representing standards or samples) bound to the green tips of the immobilized antibodies. A black arrow points down from the text to the diagram.
3. Wash 4 times  
Add 100  $\mu$ L Detection Antibody solution  
Incubate 1 hr., RT, shaking



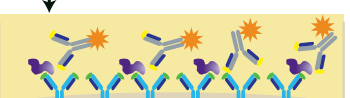
The diagram shows the well with the purple sample molecules and the blue immobilized antibodies. Now, grey Y-shaped structures (representing the detection antibody) are bound to the green tips of the immobilized antibodies. A black arrow points down from the text to the diagram.
4. Wash 4 times  
Add 100  $\mu$ L Avidin-HRP A solution  
Incubate 30 min, RT, shaking



The diagram shows the well with the purple sample molecules, the blue immobilized antibodies, and the grey detection antibodies. Now, orange star-shaped structures (representing Avidin-HRP) are bound to the green tips of the detection antibodies. A black arrow points down from the text to the diagram.
5. Wash 5 times  
Add 100  $\mu$ L Substrate Solution F  
Incubate 15 min, RT, in the dark



The diagram shows the well with the purple sample molecules, the blue immobilized antibodies, the grey detection antibodies, and the orange Avidin-HRP molecules. Now, the well is filled with a light purple liquid (representing the substrate solution). A black arrow points down from the text to the diagram.
6. Add 100  $\mu$ L Stop Solution



The diagram shows the well with the purple sample molecules, the blue immobilized antibodies, the grey detection antibodies, the orange Avidin-HRP molecules, and the light purple substrate. Now, the well is filled with a light yellow liquid (representing the stop solution). A black arrow points down from the text to the diagram.
7. Read absorbance at 450 nm and 570 nm

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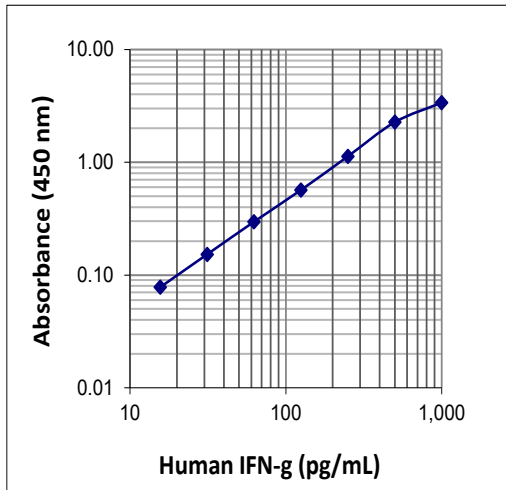
## Calculation of Results:

The data can be best calculated with computer-based curve-fitting software using a 5- or 4-parameter logistics curve-fitting algorithm. If an appropriate software is not available, use log-log graph paper to determine sample concentrations. Determine the mean absorbance for each set of duplicate or triplicate standards, controls, and samples. Plot the standard curve on log-log graph paper with cytokine concentration on the X-axis and absorbance on the Y-axis. Draw a best fit line through the standard points. To determine the unknown cytokine concentrations, find the mean absorbance value of the unknown concentration on the Y-axis and draw a horizontal line to the standard curve. At the point of intersection, draw a vertical line to the X-axis and read the cytokine concentration.

If samples were diluted, multiply the concentration by the appropriate dilution factor. If a test sample's absorbance value falls outside the linear portion of the standard curve, the test sample needs to be re-analyzed at a higher (or lower) dilution as appropriate.

## Typical Data:

This standard curve was generated at BioLegend for demonstration purposes only. A standard curve must be run with each assay.



**Performance Characteristics:**

**Specificity:** No cross reactivity was observed when this kit was used to analyze 14 human recombinant cytokines/chemokines and mouse IFN- $\gamma$  at up to 50 ng/mL.

**Sensitivity:** The minimum detectable concentration of IFN- $\gamma$  is 5.6 pg/mL.

**Recovery:** Recombinant human IFN- $\gamma$  was spiked into 4 human serum samples at concentrations of 500 pg/mL and 125 pg/mL then analyzed with the LEGEND MAX™ Human IFN- $\gamma$  ELISA kit. On average, 95.6 % of the cytokine was recovered from the serum samples.

**Linearity:** Four human serum samples spiked with high concentrations of IFN- $\gamma$  were diluted with the appropriate matrix to produce samples with concentrations within the dynamic range and then assayed. On average, 98.5% of the expected cytokine was detected from the serum samples.

**Intra-Assay Precision:** Sixteen replicates of each of two samples containing different IFN- $\gamma$  concentrations were tested in one assay.

Concentration	Sample 1	Sample 2
Number of Replicates	16	16
Mean Concentration (pg/mL)	248.3	74.2
Standard Deviation	6.8	4
%CV	2.7	5.4

**Inter-Assay Precision:** Two samples containing different concentrations of IFN- $\gamma$  were tested in four independent assays.

Concentration	Sample 1	Sample 2
Number of Assays	4	4
Mean Concentration (pg/mL)	237.6	70.5
Standard Deviation	16.6	7.1
%CV	7.0	10.1

**Biological Samples:** Freshly isolated human peripheral blood mononuclear cells at a concentration of  $1 \times 10^6$  cells/mL were stimulated with 10  $\mu$ g/mL of plate-coated anti-CD3 antibody and 1  $\mu$ g/ml of soluble anti-CD28 antibody at 37°C for 24 and 48 hours. Cell culture supernatants were collected and assayed for concentrations of natural human IFN- $\gamma$ . The resulting human IFN- $\gamma$  concentration averaged 6,004 pg/mL and 10,200 pg/mL in anti-CD3 and anti-CD28 double-stimulated cell supernatant on day 1 and day 2 respectively. It was undetectable in the unstimulated samples.

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**Troubleshooting Guide:**

Problem	Probable Cause	Solution
High Background	Background wells were contaminated	Avoid cross-well contamination by using the provided plate sealers. Use multichannel pipettes and change tips between pipetting samples and reagents.
	Insufficient washes	Increase number of washes. Increase soaking time between washes prior to addition of substrate solution.
	TMB Substrate Solution was contaminated	TMB Substrate Solution should be clear and colorless prior to addition to wells. Use a clean container prior to pipetting substrate solution into wells.
No or poor signal	Detection Antibody, Avidin-HRP or Substrate solution were NOT added	Rerun the assay and follow the protocol.
	Wrong reagent or reagents were added in wrong sequential order	
	Insufficient plate agitation	The plate should be agitated during all incubation steps using a plate shaker at a speed where solutions in wells are within constant motion without splashing.
	The wash buffer contains Sodium Azide (NaN <sub>3</sub> )	Avoid Sodium Azide contamination in the wash buffer as it inhibits HRP activity.
	Incubations were done at an inappropriate temperature, timing or without agitation	Rerun the assay and follow the protocol.
Low or poor standard curve signal	The standard was incorrectly reconstituted or diluted	Adjust the calculations and follow the protocol.
	Standard was inappropriately stored	Store the reconstituted standard stock solution in polypropylene vials at -70°C. Avoid repeated freeze-thaw cycles.
	Reagents added to wells with incorrect concentrations	Check for pipetting errors and the correct reagent volume.

Problem	Probable Cause	Solution
Signal is high, standard curves have saturated signal	Standard reconstituted with less volume than required	Reconstitute new lyophilized standard with the correct volume of solution recommended in the protocol.
	Standards/samples, detection antibody, Avidin-HRP or substrate solution were incubated for too long	Rerun the assay and follow the protocol.
Sample readings are out of range	Samples contain no or below detectable levels of the analyte	If samples are below detectable levels, it may be possible to use a larger sample volume. Contact technical support for appropriate protocol modifications.
	Samples contain analyte concentrations greater than highest standard point	Samples may require dilution and analysis.
High variation in samples and/or standards	Multichannel pipette errors	Confirm that pipette calibrations are accurate.
	Plate washing was not adequate or uniform	Ensure pipette tips are tightly secured. Ensure uniformity in all wash steps.
	Non-homogenous samples	Thoroughly mix samples before assaying.
	Samples may have high particulate matter	Remove particulate matter by centrifugation.
	Cross-well contamination	Do not reuse plate sealers.  Always change tips for reagent additions. Ensure that pipette tips do not touch the reagents on the plate.

<b>ELISA Plate Template</b>												
	1	2	3	4	5	6	7	8	9	10	11	12
A												
B												
C												
D												
E												
F												
G												
H												





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