BioLegend®

Enabling Legendary Discovery™

LEGEND MAX™

ELISA Kit

Human IFN-ß

Cat. No. 449507

ELISA Kit for Accurate Quantitation of
Human IFN-ß
in Cell Culture Supernatant

BioLegend, Inc.
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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Introduction:

Type I Interferons (IFN-alpha/beta) are produced primarily in response to viral infection by natural IFN-producing cells (IPCs) as part of the host immune response. IFN-beta binding results in the activation of the tyrosine kinases Jak1 and Tyk2, phosphorylation of members of the STAT family of transcription factors, and the transcription and expression of the immune response genes. IFN-beta is currently used clinically for treatment of tumors, infections and multiple sclerosis.

The LEGEND MAX™ Human IFN-ß ELISA Kit is a Sandwich Enzyme-Linked Immunosorbent Assay (ELISA) with a 96-well strip plate that is pre-coated with anti-human IFN-ß antibody. The detection antibody is a biotinylated anti-human IFN-ß antibody. This kit is specifically designed for the accurate quantitation of human IFN-ß in cell culture supernatant. It is analytically validated with ready-to-use reagents.

Materials Provided:

Description	Quantity	Volume	Part #
Human IFN-ß pre-coated 96-well Strip Microplate	1 plate		750004150
Human IFN-ß Detection Antibody	1 bottle	12 mL	750004152
Human IFN-ß Lyophilized Standard	1 vial	Lyophilized	750003799
Streptavidin-Polymer HRP	1 bottle	12 mL	750002513
Assay Buffer B	1 bottle	25 mL	79128
Wash Buffer (20x)	1 bottle	50 mL	78233
Substrate Solution D	1 bottle	12 mL	78115
Stop Solution	1 bottle	12 mL	79133
Plate Sealers	4 sheets		78101

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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
- Polypropylene 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.

<table>
<tr><th colspan="2">Opened or Reconstituted Components</th></tr>
<tr><td>Microplate wells</td><td>If not all microplate strips are 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.</td></tr>
<tr><td>Standard</td><td>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.</td></tr>
<tr><td>Streptavidin-Polymer HRP</td><td rowspan="5">Store opened reagent bottles at 2° - 8°C and use within 1 month</td></tr>
<tr><td>Assay Buffer B</td></tr>
<tr><td>Wash Buffer (20X)</td></tr>
<tr><td>Substrate Solution D</td></tr>
<tr><td>Stop Solution</td></tr>
</table>

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).
2. Substrate Solution D is harmful if inhaled or ingested. Avoid skin, eye and clothing contact.

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°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-ß Lyophilized Standard by adding the volume of Assay Buffer B to make the 21 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. For cell culture supernatant samples, the end user may need to determine the dilution factors in a preliminary experiment. Serum and plasma samples have not been validated.

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 not all microplate strips will be used, remove the excess strips by press-

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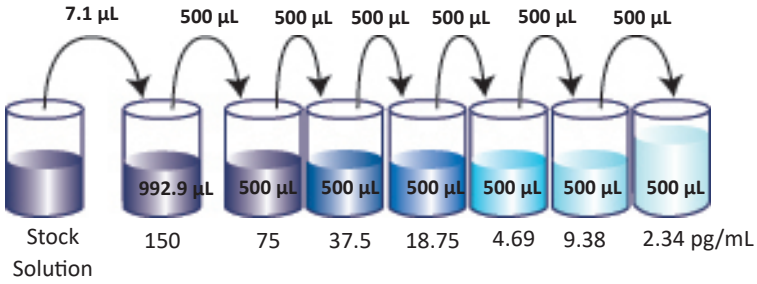
ing up from underneath each strip. Place excess strips back in the foil pouch with the included desiccant pack and reseal.

3. Prepare 1000 µL of the 150 pg/mL top standard by adding 7.1 uL of the 21 ng/mL standard stock solution into 992.9 µL Assay Buffer B. Perform six two-fold serial dilutions of the 150 pg/mL top standard in separate tubes using Assay Buffer B as the diluent. Thus, the human IFN-ß standard concentrations in the tubes are 150 pg/mL, 75 pg/mL, 37.5 pg/mL, 18.75 pg/mL, 9.38 pg/mL, 4.69 pg/mL and 2.34 pg/mL, respectively. Assay Buffer B serves as the zero standard (0 pg/mL).



4. Wash the plate 4 times with at least 300 µL 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 µL of Assay Buffer B to each well that will contain either standard dilutions or samples. Then add 50 µL of standard dilutions or samples to the appropriate wells.

6. Seal the plate with a Plate Sealer included in the kit and incubate the plate for 2 hours at room temperature with shaking.

7. Discard the contents of the plate into a sink, then wash the plate 4 times with 1X Wash Buffer as in step 4.

8. Add 100 µL of Human IFN-ß Detection Antibody solution to each well, seal the plate and incubate at room temperature for 1 hour while shaking.

9. Discard the contents of the plate into a sink, then wash the plate 4 times with 1X Wash Buffer as in step 4.

10. Add 100 µL of Polymer solution to each well, seal the plate and incubate at room temperature for 30 minutes while shaking.

11. 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.

12. (see note below.)* Add 100 µL of Substrate Solution D to each well and incubate for 10 minutes in the dark. Wells containing Human IFN-ß should turn blue in color with an intensity proportional to its concentration. It is not necessary to seal the plate during this step.
    ****Note**: Optimal substrate incubation time depends on labratory conditions and the optimal linear ranges of ELISA plate readers.

13. Stop the reaction by adding 100 µL of Stop Solution to each well. The solution color should change from blue to yellow.

14. 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

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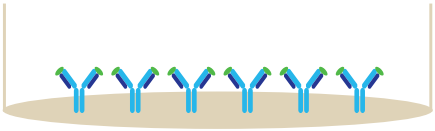
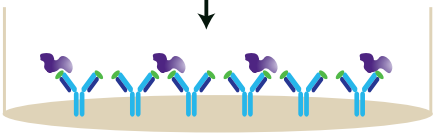
Assay Procedure Summary

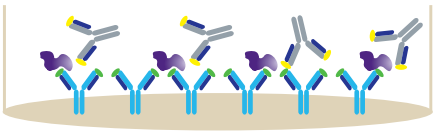
1. Wash 4 times.
Add 50 µL Assay Buffer B to standard wells and sample wells



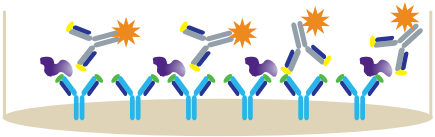
2. Add 50 µL of standard or sample, incubate 2 hr, RT, shaking



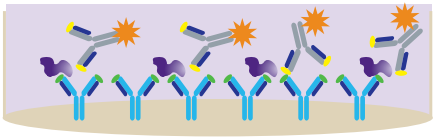
3. Wash 4 times
Add 100 µL of Human IFN-ß Detection Antibody solution.
Incubate 1 hr, RT, shaking



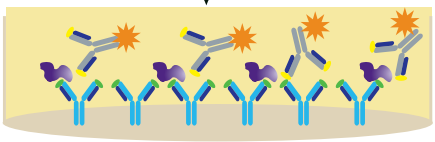
4. Wash 4 times
Add 100 µL Polymer solution.
Incubate 30 min, RT, shaking



5. Wash 5 times with soaking (see note below.)* Add 100 µL Substrate Solution D. Incubate 10 mins **, RT, in the dark.
**Note: Optimal substrate incubation time depends on labratory conditions and the optimal linear ranges of ELISA plate readers.



6. Add 100 µL Stop Solution



7. Read absorbance at 450 nm and 570 nm

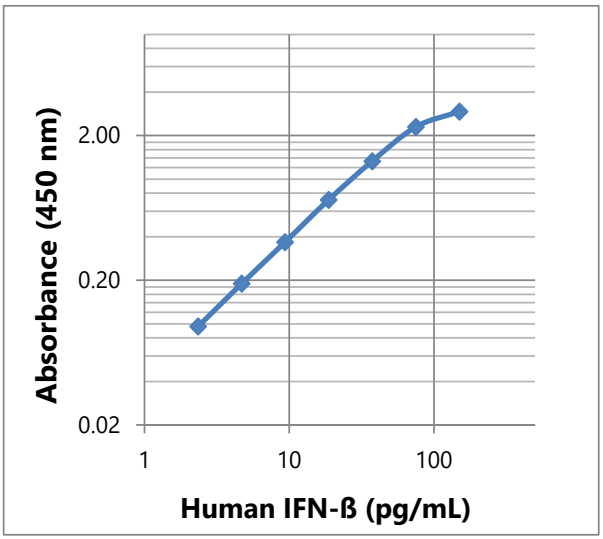
Calculation of Results:

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 analyte concentration on the X-axis and absorbance on the Y-axis. Draw a best fit line through the standard points. To determine the unknown analyte 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 analyte 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: This kit recognizes natural and recombinant Human IFN-ß. No cross reactivity was observed when this kit was used to analyze the following recombinant proteins at 50 ng/mL.

Human	IL-6, IFNγ, IFNα2
Mouse	IFNß1

Sensitivity: The minimum detectable concentration of Human IFN-ß is 0.91 ± 0.16 pg/mL pg/mL (n=6).

Recovery: Recombinant Human IFN-ß at 3 different concentrations was spiked into RPMI + 10% FCS. Sample recovery was then analyzed with the LEGEND MAX™ Human IFN-ß Kit.

Sample Type	N	% Recovery
Cell Culture Supernatant	1	92%

Linearity: A549 human lung carcinoma cells were stimulated with poly I:C in the presence of Lipofectamine. The stimulated cell culture supernatant was serially diluted with ABB following the linear range in order to determine the dilutional linearity.

Sample Type	N	% Linearity
Cell Culture Supernatant	1	108%

Intra-Assay Precision: Two samples containing different human IFN-ß concentrations were tested on one plate with 16 replicates.

Concentration	Sample 1A	Sample 2A
Number of Replicates	16	16
Mean Concentration (pg/mL)	65.2	8.2
Standard Deviation	2.8	0.4
%CV	4.2%	4.6%

Inter-Assay Precision: Two samples containing different human IFN-ß concentrations were tested in ten independent assays.

Concentration	Sample 1	Sample 2
Number of Assays	10	10
Mean Concentration (pg/mL)	75.8	8.3
Standard Deviation	7.2	0.5
%CV	9.5%	6.3%

Biological Samples: A549 human lung carcinoma cells were left untreated or treated with poly I:C in the presence of Lipofectamine. Aliquots of the cell culture supernates were removed and assayed for levels of human IFN-β.

	Unstimulated A549 Poly I:C	Stimulated A549 Poly I:C
pg/mL	ND	219

Troubleshooting Guide:

Problem	Probable Cause	Solution
Signal is high, standard curves have saturated absorbance	Standard reconstituted with less volume than required	Reconstitute lyophilized standard with correct volume of solution recommended in the protocol.
	Plate incubation was too long	Decrease incubation time.
	Detection antibody incubation time is too long	Decrease detection antibody incubation time.
	Avidin-HRP incubation time is too long.	Decrease Avidin-HRP incubation time.
	Substrate solution incubation time is too long	Decrease substrate solution incubation time.
Sample readings are out of range	Samples contain analyte concentrations greater than highest standard point.	Samples may require dilution and reanalysis.
High variation in samples and/or standards	Multichannel pipette errors	Calibrate the pipettes.
	Plate washing was not adequate or uniform	Make sure pipette tips are tightly secured. Confirm all reagents are removed completely in all wash steps.
	Non-homogenous samples	Thoroughly mix samples before pipetting.
	Samples may have high particulate matter	Remove the particulate matter by centrifugation.
	Insufficient plate agitation	The plate should be agitated during all incubation steps using an ELISA plate shaker at a speed where solutions in wells are within constant motion without splashing.
	Cross-well contamination	When reusing plate sealers check that no reagent has touched the sealer. Care should be taken when using the same pipette tips used for reagent additions. Ensure that pipette tips do not touch the reagents on the plate.

Problem	Probable Cause	Solution
Background is high	Background wells were contaminated	Avoid cross-well contamination by using the sealer appropriately. Use multichannel pipettes without touching the reagents on the plate.
	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 signal	Incorrect or no Detection Antibody was added	Add appropriate Detection Antibody and continue.
	Avidin-HRP was not added. Substrate solution was not added.	Add Avidin-HRP according to protocol and continue. Add substrate solution and continue.
	Wash buffer contains sodium azide	Avoid sodium azide in the Wash Buffer.
Low or poor signal for the standard curve	Standard was incompletely reconstituted or was inappropriately stored	Reconstitute standard according to protocol. Store reconstituted standard in appropriate vials. Store reconstituted standard at -70°C.
	Reagents added to wells with incorrect concentrations	Check for pipetting errors and correct reagent volume.
	Incubations done at inappropriate temperature, timing or agitation	Assay conditions need to be checked.

ELISA Plate Template

	1	2	3	4	5	6	7	8	9	10	11	12
A												
B												
C												
D												
E												
F												
G												
H												

Legend Max™ Kits are manufactured by BioLegend Inc.
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