BioLegend®

Enabling Legendary Discovery™

Human IFN-α2

ELISA MAX™ *Deluxe Set*

Cat. No. 446404

BioLegend's ELISA MAX™ Deluxe Sets contain the components necessary for the accurate quantification of natural and recombinant human IFN-α2. These sets are designed for cost-effective and accurate quantification of free human IFN-α2 in cell culture supernatant, serum, plasma or other biological fluids. They are sensitive, accurate, and robust.

It is highly recommended that this instruction sheet be read in its entirety before using this product. Do not use this set beyond the expiration date.

Materials Provided

1. Human IFN-α2 ELISA MAX™ Capture Antibody (200X)
2. Human IFN-α2 ELISA MAX™ Detection Antibody (200X)
3. Human IFN-α2 Standard
4. Matrix Diluent A
5. Avidin-HRP (1000X)
6. Substrate Solution F
7. Coating Buffer B (5X)
8. Assay Diluent A (5X)
9. Assay Buffer G

Introduction

Interferon alpha-2 (IFN-α2) is a cytokine belonging to the Type 1 IFN family. IFN-α2 can act on all cell types due to the ubiquitous expression of the Type 1 IFN receptor (IFNAR). IFN-α2 acts to combat infection by stimulating the production of proteins that inhibit viral replication and prevent viral entry into neighboring cells. IFN-α2 can stimulate the immune system by initiating T and NK cell mediated cytotoxicity. IFN-α2 also can inhibit cancer cell proliferation and activate immune system to target cancer cells. IFN-α2 has been used therapeutically in a variety of viral infections and certain cancers.

Principle of the Test

BioLegend's ELISA MAX™ Deluxe Set is a sandwich Enzyme-Linked Immunosorbent Assay (ELISA).

A mouse monoclonal anti-human IFN-α2 is first coated on a 96-well plate. Standards and samples are added to the wells, and IFN-α2 binds to the immobilized capture antibody. Next, a biotinylated mouse monoclonal anti-human IFN-α2 detection antibody is added, producing an antibody-antigen-antibody "sandwich". Avidin-horseradish peroxidase is subsequently added, followed by TMB Substrate Solution, producing a blue color in proportion to the concentration of IFN-α2 present in the sample. Finally, the Stop Solution changes the reaction color from blue to yellow, and the microwell absorbance is read at 450 nm with a microplate reader.

For research purposes only. Not for use in diagnostic or therapeutic procedures.

Materials to be Provided by the End-User

- Microwell plates: BioLegend Cat. No. 423501 is recommended
- Wash Buffer: BioLegend Cat. No. 421601 is recommended, or PBS + 0.05% Tween-20
- Stop Solution: BioLegend Cat. No. 423001 is recommended, or acid solution, e.g. 2N H_2SO_4
- Plate Sealers: BioLegend Cat. No. 423601 is recommended
- PBS (Phosphate-Buffered Saline): 8.0 g NaCl, 1.16 g Na_2HPO_4, 0.2 g KH_2PO_4, 0.2 g KCl, add deionized water to 1 L; pH to 7.4, 0.2 µm filtered
- Deionized (DI) water
- A microplate reader capable of measuring absorbance at 450 nm
- Adjustable pipettes to measure volumes ranging from 2 µL to 1 mL
- Wash bottle or automated microplate washer
- Log-Log graph paper or software for data analysis
- Tubes to prepare standard dilutions
- Timer
- Absorbent paper

Storage Information

- Store kit components between 2°C and 8°C.
- After reconstitution of the lyophilized standard with Assay Buffer G, aliquot into polypropylene vials and store at -70°C for up to one month. Avoid repeated freeze/thaw cycles.
- Prior to use, bring all components to room temperature (18°C-25°C). Upon assay completion, return all components to appropriate storage conditions.

Health Hazard Warnings

1. Reagents that contain preservatives may be harmful if ingested, inhaled or absorbed through the skin. Refer to the MSDS online for details (www.biolegend.com/msds).
2. Substrate Solution F is harmful if ingested. Additionally, avoid skin, eye or clothing contact.
3. To reduce the likelihood of blood-borne transmission of infectious agents, handle all serum and/or plasma in accordance with NCCLS regulations.

Specimen Collection and Handling

Cell Culture Supernatant: If necessary, centrifuge to remove debris prior to analysis. Samples can be stored at < -20°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 X *g*. Remove serum layer and assay immediately or store serum samples at < -20°C. Avoid repeated freeze/thaw cycles. Serum specimens should be clear and non-hemolyzed.

Plasma: Collect blood samples in citrate, heparin or EDTA containing tubes. Centrifuge for 20 minutes at 1,000 x g within 30 minutes of collection. Assay immediately or store plasma samples at < -70°C. Avoid repeated freeze-thaw cycles.

Reagent and Sample Preparation

Do not mix reagents from different sets or lots. Reagents and/or antibodies from different manufacturers should not be used with this set. All reagents should be diluted immediately prior to use.

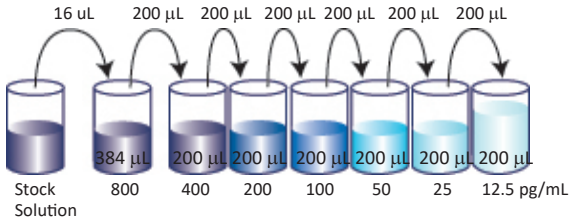
NOTE: Precipitation of 5X Assay Diluent A may be observed when stored long term between 2°C and 8°C. The precipitation does not alter the performance of the Buffer. If heavy precipitation is observed after the dilution to 1X Assay Diluent A, it can be filtered to clarify the solution.

Preparation of 1X Reagent for 1 Plate

Material	Dilute with
2.4 mL of Coating Buffer B (5X)	9.6 mL of Deionized Water
60 µL of Capture Antibody (200X)	12 mL of 1X Coating Buffer B
12 mL of Assay Diluent A (5X)	48 mL of PBS
60 µL of Detection Antibody (200X)	12 mL of 1X Assay Diluent A
12 µL of Avidin-HRP (1,000X)	12 mL of 1X Assay Diluent A

Lyophilized vials are under vacuum pressure. Reconstitute lyophilized standard by following the instructions described in Lot-Specific Certificate of Analysis/ELISA MAX™ Deluxe Set Protocol to generate a 20 ng/mL stock solution. Allow the reconstituted standard to sit for 15 minutes at room temperature, then mix gently prior to making dilutions.

Prepare 400 µL of the top standard at a concentration of 800 pg/mL with 16 µL of the 20 ng/mL stock solution and 384 µL Assay Buffer G. Perform six two-fold serial dilutions of the 800 pg/mL top standard with Assay Buffer G in separate tubes. After diluting, the human IFN-α2 standard concentrations are 800, 400, 200, 100, 50, 25, and 12.5 pg/mL, respectively. Assay Buffer G serves as the zero standard (0 pg/mL).



Samples: In general, serum samples require a 2-fold dilution and plasma samples require a 4-fold dilution. Tissue culture supernatants do not require dilution. If dilution is required, use Assay Buffer G.

Assay Procedure

Do not use sodium azide in any solutions as it inhibits the activity of the horseradish-peroxidase enzyme.

1. One day prior to running the ELISA, dilute Capture Antibody in 1X Coating Buffer B as described in Reagent Preparation. Add 100 µL/well of this Capture Antibody solution to the 96-well plate provided in this set. Seal plate and incubate overnight between 2°C and 8°C.
2. Bring all reagents to room temperature (RT) 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.
3. Wash plate 4 times with at least 300 µL Wash Buffer per well and blot residual buffer by firmly tapping plate upside down on absorbent paper. **All subsequent washes should be performed similarly.**
4. To block non-specific binding and reduce background, add 200 µL 1X Assay Diluent A per well.
5. Seal plate and incubate at RT for 1 hour with shaking on a plate shaker (e.g. 500 rpm with a 0.3 cm circular orbit). All subsequent incubation with shaking should be performed similarly.

6. While plate is being blocked, prepare the appropriate sample dilutions (if necessary) and standards.
7. Wash plate 4 times with Wash Buffer.
8. a) If reading serum or plasma samples, add 50 µL of Matrix Diluent A to wells that will contain standards. If reading cell culture samples, add 50 uL of Assay Buffer G to wells that will contain standards.
   b) Add 50 µL of Assay Buffer G to each well that will contain samples.
   c) Add 50 µL of standards or samples to the appropriate wells.
9. Seal plate, incubate at RT for 2 hours with shaking.
10. Wash plate 4 times with Wash Buffer.
11. Add 100 µL/well of diluted Detection Antibody solution, seal plate and incubate at RT for 1 hour with shaking.
12. Wash plate 4 times with Wash Buffer.
13. Add 100 µL/well of diluted Avidin-HRP solution, seal plate and incubate at RT for 30 minutes with shaking.
14. Wash plate 5 times with Wash Buffer. For this final wash, soak wells in Wash Buffer for 30 seconds to 1 minute for each wash. This will help minimize background.
15. Add 100 µL/well Substrate Solution F and incubate **in the dark** for 15 minutes. Positive wells should turn blue in color. It is not necessary to seal the plate during this step.
16. Stop reaction by adding 100 µL/well of Stop Solution. Positive wells should turn from blue to yellow.
17. Read absorbance at 450 nm within 15 minutes. If the reader can read at 570 nm, the absorbance at 570 nm can be subtracted from the absorbance at 450 nm.

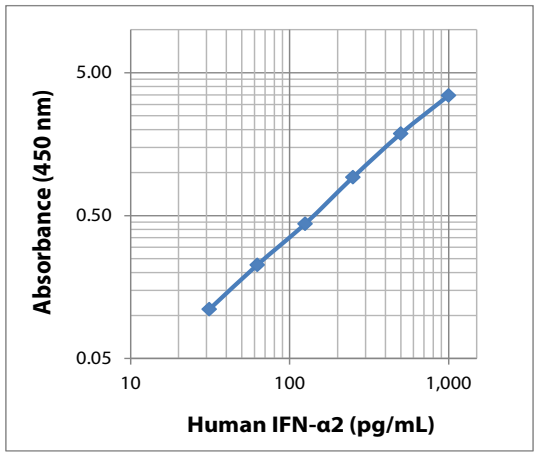
***Optimal substrate incubation time depends on laboratory conditions and the optical linear ranges of ELISA plate readers.**

Calculation of Results

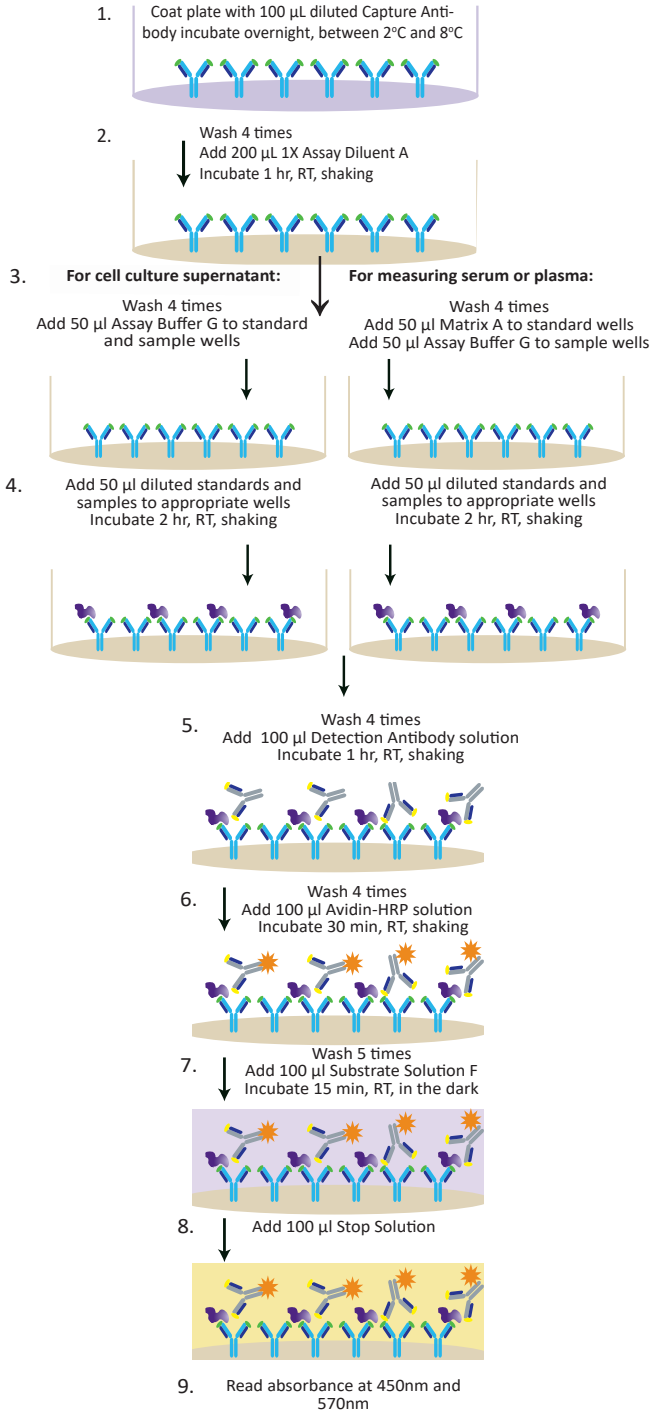
The data is best calculated with computer-based curve-fitting software using a 5- or 4-parameter logistics curve-fitting algorithm. If the samples were diluted, multiply by the appropriate dilution factor. If a test sample's absorbance value falls outside the standard curve ranges, that test sample needs to be reanalyzed at a higher or lower dilution as appropriate.

Typical Data

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



Assay Procedure Summary



Performance Characteristics

Sensitivity: The expected minimum detectable concentration of human for this set is 2.94 pg/mL with assay buffer and 2.57 pg/mL with matrix.

Specificity: This set recognizes natural and recombinant IFN-α2. Kit has equal recognition between the IFN-α2 allelic variants IFN-α2a, IFN-α2b, and IFN-α2c. There is 100% cross reactivity with IFN-α6.

Troubleshooting

High Background:

- Background wells were contaminated.
- Matrix used had endogenous analyte.
- Plate was insufficiently washed.
- TMB Substrate Solution was contaminated.

No signal:

- Incorrect or no antibodies were added.
- Avidin-HRP was not added.
- Substrate solution was not added.
- Wash buffer contains sodium azide.

Low or poor signal for the standard curve:

- Standard was incompletely reconstituted or was stored improperly.
- Reagents were added to wells with incorrect concentrations.
- Plate was incubated with improper temperature, timing or agitation.

Signal too high, standard curves saturated:

- Standard was reconstituted with less volume than required.
- One or more reagent incubation steps were too long.
- Plate was incubated with inappropriate temperature, timing, or agitation.

Sample readings out of range:

- Samples contain no or below detectable levels of analyte.
- Samples contain analyte concentrations greater than highest standard point.

High variations in samples and/or standards:

- Pipetting errors may have occurred.
- Plate washing was inadequate or non-uniform.
- Samples were not homogenous.
- Samples or standard wells were contaminated.

BioLegend, Inc.

BioLegend is ISO 13485 Certified.

8999 BioLegend Way, San Diego, CA 92121

Tel: 1-858-768-5800, Fax: 1-877-455-9587

www.biolegend.com

For other technical resources, please visit: **www.biolegend.com/en-us/support** or email: **techserv@biolegend.com**