Mouse IFN-γ ELISA MAX™ Standard Sets

Cat. No. 430801 (5 plates)  
430802 (10 plates)  
430803 (20 plates)

BioLegend's ELISA MAX™ Standard Sets contain the capture and detection antibodies, recombinant protein standard, and Avidin-HRP required for the accurate quantification of natural and recombinant mouse IFN-γ. These sets are cost-effective and designed for experienced ELISA users. Optimization of reagent concentrations and assay conditions may be required. It is highly recommended that the instruction sheet be read in its entirety before using this product. Use the recommended assay protocol, microwell plates, buffers, diluent, and substrate solution to obtain desired assay results. Do not use this set beyond the expiration date.

Materials Provided
1. Mouse IFN-γ ELISA Capture Antibody (200X)  
2. Mouse IFN-γ ELISA Detection Antibody (200X)  
3. Mouse IFN-γ Standard  
4. Avidin-HRP (1000X)  
5. Instruction Sheet  
6. Lot-Specific Instruction/Analysis Certificate

Introduction
Mouse IFN-γ is a potent multifunctional cytokine which is secreted primarily by activated NK cells and T cells. Originally characterized based on anti-viral activities, IFN-γ also exerts anti-proliferative, immunoregulatory, and proinflammatory activities. IFN-γ can upregulate MHC class I and II antigen expression by antigen-presenting cells.

Principle of the Test
BioLegend’s ELISA MAX™ Standard Set is a sandwich Enzyme-Linked Immunosorbent Assay (ELISA).

Calculation of Results
Plot the standard curve on log-log axis 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 in the samples, find the absorbance value of the unknown 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 corresponding cytokine concentration. If the samples were diluted, multiply by the appropriate dilution factor. The data is best calculated with computer-based curve-fitting software using a 5- or 4-parameter logistics curve-fitting algorithm. 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.

![Standard Curve Graph](image)

Performance Characteristics

**Specificity:** No cross reactivity was observed when this kit was used to analyze multiple human, mouse and rat recombinant proteins.

References Using This Set

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 contained 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 inappropriate 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 nonuniform.
- Samples were not homogeneous.
- Samples or standard wells were contaminated.

For other technical resources, please visit: [www.biolegend.com/support](http://www.biolegend.com/support) or email: techserv@biolegend.com
**Materials to be Provided by the End-User**

- Microwell plates: 96-well Nunc MaxiSorp™ is recommended.
- A microplate reader capable of measuring absorbance at 450 nm
- Adjustable pipettes to measure volumes ranging from 2 μL to 1 mL
- Deionized (DI) water
- Coating Buffer: 8.4 g NaHCO₃, 3.56 g Na₂CO₃, add DI H₂O to 1.0 L, pH to 9.5 (BioLegend Cat. No. 421701 is recommended.)
- Assay Diluent: 10% Fetal Bovine Serum or 1% BSA in Phosphate-Buffered Saline (PBS) (BioLegend Cat. No. 421203 is recommended.)
- PBS: 8.0 g NaCl, 1.16 g Na₂HPO₄, 0.2 g KH₂PO₄, 0.2 g KCl, add DI water to 1.0 L, pH to 7.4
- Wash Buffer: Phosphate-Buffered Saline (PBS) + 0.05% Tween-20 (BioLegend Cat. No. 421601 is recommended.)
- Wash bottle or automated microplate washer
- TMB Substrate Solution – BioLegend Cat. No. 421101 is recommended.
- Stop Solution (2 N H₂SO₄)
- Log-Log graph paper or software for data analysis
- Tubes to prepare standard dilutions
- Timer
- Absorbent paper

**Storage Information**

- Store kit components at 4°C.
- Prior to use, bring all components to room temperature
- 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/support/#msds).
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**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/support/#msds).
2. TMB substrate solution 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 sample in a citrate, heparin or EDTA containing tube. Centrifuge for 10 minutes at 1,000 X g within 30 minutes of collection. Assay immediately or store plasma samples at -20°C. Avoid repeated freeze/thaw cycles. Plasma specimens should be clear and non-hemolyzed.

**Reagent Preparation**

**Do not mix reagents from different sets or lots. Avidin-HRP, Mouse IFN-γ Standard, and/or antibodies from different manufacturers should not be used with this set. All reagents should be diluted immediately prior to use.**

1. Dilute the pre-titrated Capture Antibody 1:200 in Coating Buffer. For one plate, dilute 60 μL Capture Antibody in 11.94 mL Coating Buffer.
2. Reconstitute the lyophilized standard with 0.2 mL of Assay Diluent, re-cap vial, and mix well. Allow the reconstituted standard to sit for 15 minutes at room temperature, then invert/vortex to mix.
3. Prior to use, prepare 1,000 μL of the top standard at a concentration of 1,000 pg/mL from stock solution in Assay Diluent (refer to Lot-Specific Instruction/Analysis Certificate).
4. Dilute the pre-titrated Biotinylated Detection Antibody 1:200 in Assay Diluent. For one plate, dilute 60 μL Detection Antibody in 11.94 mL Assay Diluent.
5. Dilute Avidin-HRP 1:1000 in Assay Diluent. For one plate, dilute 12 μL Avidin-HRP in 11.99 mL Assay Diluent.
6. Prepare all other reagents required for the assay including TMB Substrate Solution. Refer to reagent description in the section “Materials to be Provided by the End-User”.

**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 Coating Buffer. Add 100 μL of this Capture Antibody solution to all wells of a 96-well plate provided in the set. Seal plate and incubate overnight at 4°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 Assay Diluent per well.
5. Seal plate and incubate at RT for 1 hour with shaking at 200 rpm on a plate shaker.
6. While plate is being blocked, prepare standard dilutions and appropriate sample dilutions (if necessary).
7. Prepare 3,000 μL of top standard at 1,000 pg/mL from stock solution in Assay Diluent (refer to Lot-Specifications/Analysis Certificate). Perform six two-fold serial dilutions of the 1,000 pg/mL top standard in separate tubes as shown below. After diluting, the mouse IFN-γ standard concentrations are 1,000 pg/mL, 500 pg/mL, 250 pg/mL, 125 pg/mL, 62.5 pg/mL, 31.25 pg/mL, and 15.6 pg/mL, respectively. 1X Assay Diluent serves as the zero standard (0 pg/mL).
8. Wash plate 4 times with Wash Buffer.
9. Add 100 μL/well of standard dilutions and samples to the appropriate wells. If needed, samples can be further diluted with Assay Diluent before adding 100 μL/well diluted samples.
10. Seal plate and incubate at RT for 2 hours with shaking.
11. Wash plate 4 times with Wash Buffer.
12. Add 100 μL of diluted Detection Antibody solution to each well, seal plate and incubate at RT for 1 hour with shaking.
13. Wash plate 4 times with Wash Buffer.
14. Add 100 μL of diluted Avidin-HRP solution to each well, seal plate and incubate at RT for 30 minutes with shaking.
15. 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.
16. Add 100 μL of TMB Substrate Solution and incubate in the dark for 20-30 minutes or until the desired color develops*. Positive wells should turn blue in color. It is not necessary to seal the plate during this step.
17. Stop reaction by adding 100 μL of Stop Solution to each well. Positive wells should turn from blue to yellow.
18. Read absorbance at 450 nm within 30 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.

**Assay Procedure Summary**

**Day 1**

Add 100 μL diluted Capture Antibody solution to each well, incubate overnight at 4°C

**Day 2**

1. Wash plate 4 times
2. Add 200 μL Assay Diluent to block, incubate at room temperature for 1 hour with shaking
3. Wask plate 4 times
4. Add diluted standards and samples to the appropriate wells, incubate at room temperature for 2 hours with shaking
5. Wash plate 4 times
6. Add 100 μL diluted Detection Antibody solution to each well, incubate at room temperature for 1 hour with shaking
7. Wash plate 4 times
8. Add 100 μL diluted Avidin-HRP solution to each well, incubate at room temperature for 30 minutes with shaking
9. Wash plate 5 times, soaking for 30 seconds to 1 minute per wash
10. Add 100 μL of TMB Substrate Solution to each well, incubate in the dark for 20-30 minutes or until the desired color develops
11. Add 100 μL Stop Solution to each well
12. Read absorbance at 450 nm and 570 nm

For more information about BioLegend ELISA MAX™ Sets and LEGEND MAX™ ELISA Kits with precoated plates, visit www.biolegend.com.