LEGEND MAX™ Human IFN-γ ELISA Kit

Certificate of Analysis

Product Name: LEGEND MAX™ Human IFN-γ ELISA Kit

Product Cat. No: 430107

Lot No: B353337

Expiration Date: 07-JAN-2024

Materials Provided:

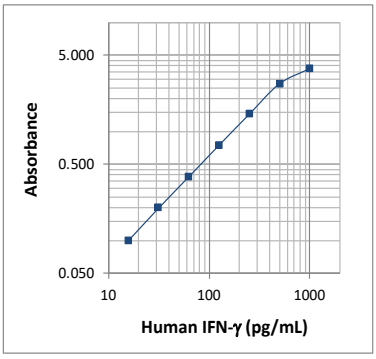
Description	Quantity	Volume (per bottle)	Part No.	Lot No.
Anti-Human IFN-γ Pre-coated 96-well Strip Microplate	1 plate	-	78213	B353343
Anti-Human IFN- γ Detection Antibody	1 bottle	12 mL	78318	B367381
Human IFN-γ Standard	1 vial	20.8 ng	79103	B361698
Avidin-HRP A	1 bottle	12 mL	79131	B367382
Assay Buffer A	1 bottle	25 mL	78232	B354236
Wash Buffer (20X)	1 bottle	50 mL	78233	B354244
Substrate Solution F	1 bottle	12 mL	79132	B354768
Stop Solution	1 bottle	12 mL	79133	B360740
Plate Sealers (4 sheets)	1 pack	-	78101	B366114

Storage Conditions:

1. Unopened kit: Store kit between 2°C and 8°C. Do not use this kit beyond its expiration date.
2. Opened or reconstituted components:
   - 2.1. Microplate wells: 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.
   - 2.2. 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.
   - 2.3. Other components: Store opened reagents between 2°C and 8°C and use within one month.

Standard Range: 15.6 - 1,000 pg/mL

Lot #: B353337



This standard curve is for demonstrative purposes only.

This is to certify that the product was manufactured under stringent process controls to ensure lot to lot consistency and complete lot traceability. The product has been tested and meets quality control specifications.

Signature:_____(Quality Control) Date: 07/13/2022

BioLegend is ISO 13485 certified.

FOR RESEARCH USE ONLY

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Kit Protocol

Reagent and Sample Preparation

Reagent	Preparation
Wash Buffer (1X)	Dilute 50 mL of Wash Buffer (20X) in 950 mL DI H_2O

Standard: Reconstitute the lyophilized Human IFN-γ Standard by adding 1.04 mL of Assay Buffer A to make the 20 ng/mL standard stock solution. Allow the reconstituted standard to sit at room temperature for 15-20 minutes, then briefly vortex to mix completely.

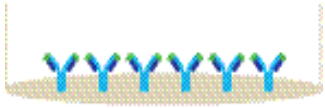
Prepare 500 µL of the top standard at 1000 pg/mL by adding 25 µl of reconstituted lyophilized standard to 475 µl Assay Buffer A. Perform six two-fold serial dilutions of the 1000 pg/mL top standard with Assay Buffer A in separate tubes. Assay Buffer A serves as the zero standard (0 pg/mL).

Samples: For cell culture supernatant samples, the end user may need to determine the dilution factors in a preliminary experiment. Serum or plasma samples should be tested initially without any dilution. However, if dilutions are required, use Assay Buffer A as the diluent.

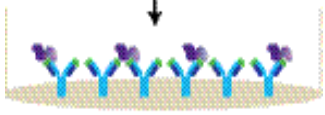
ELISA Procedure Summary

Note: 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.

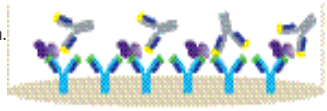
1. Wash 4 times.
   Add 50 µL Assay Buffer A.



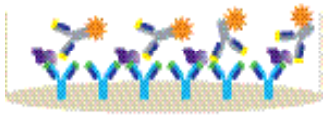
2. Add 50 µL diluted standards or samples.
   Incubate 2 hrs, RT, shaking



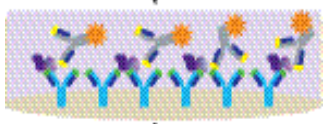
3. Wash 4 times.
   Add 100 µL Detection Antibody solution.
   Incubate 1 hr, RT, shaking.



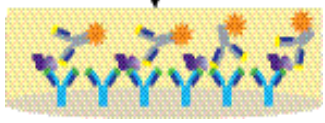
4. Wash 4 times.
   Add 100 µL Avidin-HRP A solution.
   Incubate 30 mins, RT, shaking.



5. Wash 5 times.
   Add 100 µL Substrate Solution F.
   Incubate 15 mins, RT, in the dark.



6. Add 100 µL Stop Solution.



7. Read absorbance at 450 nm and 570 nm.

Caution: Stop solution contains strong acid. wear eye, hand, and face protection.

Plate washing step is crucial to assay precision. Washing is typically repeated 4-5 times between each step to remove unbound material. Wash the plate with at least 300 µL of 1X Wash Buffer per well and blot any residual buffer by firmly tapping the plate upside down on clean absorbent paper. For the final wash, soak wells in 1X Wash Buffer for 30 seconds to 1 minute for each wash. This will help minimize background.

For more detailed set information, please refer to the online manual at: www.biolegend.com/media_assets/pro_detail/datasheets/430107.pdf

Part No. 77494_R03