User Guide

MILLIPLEX® Human Interferon Magnetic Bead Panel

96-Well Plate Assay

HIFN-130K
HIFN-130K-PMX9

Introduction

Interferons (IFNs) are widely expressed cytokines that have potent antiviral and antiproliferative effects. These cytokines are the first line of defense against viral infections and have important roles in immunosurveillance for malignant cells. IFNs are classified as type I, II and III IFNs, based upon the target receptors to which they bind. In humans, type I IFNs belong to a multigene family consisting of multiple IFNα subtypes but only one IFNβ, IFNε, IFNκ, and IFNω. There is only one type II IFN: IFNγ. The type III IFNs include IFNλ1, λ2 and λ3, which are also known as interleukin-29 (IL-29), IL 28A and IL-28B, respectively. Functionally, the type I and type III IFNs are induced during a viral infection and involved in host defense against viruses. IFNγ (type II) is primarily involved in the allergic response, in host defense against intracellular pathogens, and in control of tumor growth. Studies of IFNs have broadened the mechanistic understanding of cell signaling, gene transcription, and innate and acquired immunity.

The MILLIPLEX® portfolio offers the broadest selection of analytes across a wide range of disease states and species. Once the analytes of interest have been identified, you can rely on the quality that we build into each kit to produce results you can trust. In addition to the assay characteristics listed in the protocol, other performance criteria evaluated during the verification process include: cross-reactivity, dilution linearity, kit stability, and sample behavior (for example, detectability and stability).

Each MILLIPLEX® panel and kit includes:

- Quality controls (QCs) provided to qualify assay performance
- Comparison of standard (calibrator) and QC lots to a reference lot to ensure lot-to-lot consistency
- Optimized serum matrix to mimic native analyte environment
- Detection antibody cocktails designed to yield consistent analyte profiles within panel

In addition, each panel and kit meet stringent manufacturing criteria to ensure batch-to-batch reproducibility. The MILLIPLEX® Human Interferon Magnetic Bead Panel thus enables you to focus on the therapeutic potential of cytokines and the modulation of cytokine expression. Coupled with the Luminex® xMAP® platform in a magnetic bead format, you receive the advantage of ideal speed and sensitivity, allowing quantitative multiplex detection of dozens of analytes simultaneously, which can dramatically improve productivity.

The MILLIPLEX® Human Interferon Magnetic Bead Panel is part of the most versatile system available for cytokine, chemokine and growth factor research. From our single to multiplex biomarker solutions, we partner with you to design, develop, analytically verify and build the most comprehensive library available for protein detection and quantitation.

MILLIPLEX® products offer you:

- The ability to select a 9-plex premixed kit. If using tissue/cell culture supernatant samples, the recommended sample dilution is uniform for all analytes. Please review the "Sample Collection and Storage" section for tissue culture supernatant samples.
- The ability to choose any combination of analytes from our panel of 9 analytes to design a custom kit that better meets your needs.
- A convenient "all-in-one" box format that gives you the assurance that you will have all the necessary reagents you need to run your assay.

The MILLIPLEX® Human Interferon Magnetic Bead Panel is a 9-plex kit to be used for the simultaneous quantification of any or all of the following analytes in serum or plasma samples and tissue/cell lysate and culture supernatant samples: IFNα-2, IFNβ, IFNε, IFNɣ, IFNɣ Receptor 1, IFNλ1, IFNλ2, IFNλ3, IFNω.

For research use only. Not for use in diagnostic procedures.

Please read entire protocol before use.

It is important to use same assay incubation conditions throughout your study.

Principle

MILLIPLEX® products are based on the Luminex® xMAP® technology — one of the fastest growing and most respected multiplex technologies offering applications throughout the life-sciences and capable of performing a variety of bioassays including immunoassays on the surface of fluorescent-coded magnetic beads known as MagPlex®-C microspheres.

- Luminex® products use proprietary techniques to internally color-code microspheres with two fluorescent dyes. Through precise concentrations of these dyes, distinctly colored bead sets of 500-5.6 µm polystyrene microspheres or 80-6.45 µm magnetic microspheres can be created, each of which is coated with a specific capture antibody.
- After an analyte from a test sample is captured by the bead, a biotinylated detection antibody is introduced.
- The reaction mixture is then incubated with Streptavidin-PE conjugate, the reporter molecule, to complete the reaction on the surface of each microsphere.
- The following Luminex® instruments can be used to acquire and analyze data using two detection methods:
- The Luminex® analyzers, Luminex® 200™, FLEXMAP 3D®, and xMAP® INTELLIFLEX, are flow cytometry-based instruments that integrate key xMAP® detection components, such as lasers, optics, advanced fluidics and high-speed digital signal processors.
- The Luminex® analyzer (MAGPIX®), a CCD-based instrument that integrates key xMAP® capture and detection components with the speed and efficiency of magnetic beads.
- Each individual microsphere is identified and the result of its bioassay is quantified based on fluorescent reporter signals. We combine the streamlined data acquisition power of Luminex® xPONENT® acquisition software with sophisticated analysis capabilities of the new MILLIPLEX® Analyst 5.1, integrating data acquisition and analysis seamlessly with all Luminex® instruments.
- xMAP® INTELLIFLEX runs on INTELLIFLEX software for instrument control, run setup and generating high quality data with flexible output options. Data can be exported in xPONENT® style CSV files for compatibility with many existing analytical applications, or in the new, customizable INTELLIFLEX file format. The INTELLIFLEX file format is intended for flexibility and simplicity, allowing the user to freely select which data points to include and to reduce the time to analysis.

  The capability of adding multiple conjugated beads to each sample results in the ability to obtain multiple results from each sample. Open-architecture xMAP® technology enables multiplexing of many types of bioassays reducing time, labor and costs over traditional methods.

Storage Conditions Upon Receipt

- Recommended storage for kit components is 2–8 °C.
- For long-term storage, freeze reconstituted standards and controls at ≤ –20 °C. Avoid multiple (> 2) freeze/thaw cycles.
- **DO NOT FREEZE** Antibody-Immobilized Beads, Detection Antibody, and Streptavidin-Phycoerythrin.

Reagents Supplied

Store all reagents at 2–8 °C

Reagents	Volume	Quantity	Cat. No.
Human Interferon Panel Standard	Lyophilized	1 vial	HIFN-8130
Human Interferon Panel Quality Controls 1 and 2	Lyophilized	1 vial each	HIFN-6130
Serum Matrix	Lyophilized	1 vial	MXHSM-HIFN
Set of one 96-Well Plate with 2 sealers	-	1 set	-
Assay Buffer	30 mL	1 bottle	L-AB
10X Wash Buffer	60 mL	1 bottle	L-WB
Human Interferon Panel Detection Antibodies	3.2 mL	1 bottle	HIFN-1130
Streptavidin-Phycoerythrin	3.2 mL	1 bottle	L-SAPE4
Bead Diluent (not provided with premixed bead)	3.5 mL	1 bottle	LBD
Hydration Buffer	5 mL	1 bottle	L-HB
Mixing Bottle (not provided with premixed panel)	-	1 bottle	-

* Sodium azide or ProClin™ has been added to some reagents as a preservative. Although the concentrations are low, Sodium azide and ProClin™may react with lead and copper plumbing to form highly explosive metal azides. Dispose of unused contents and waste in accordance with international, federal, state, and local regulations.

** For details on which reagents ship with which analytes, see Product Ordering.

Human Interferon Panel Antibody-Immobilized Premixed Magnetic Beads

	Volume	Quantity	Cat. No.
Premixed 9-plex Beads	3.5 mL	1 bottle	HIFNPMX9-MAG

Human Interferon Antibody-Immobilized Magnetic Beads

Included Human Interferon Antibody-Immobilized Beads are dependent on customizable selection of analytes within the panel.

Bead/Analyte Name	Luminex® Magnetic Bead Region	Customizable 9 Analytes (50X concentration, 90 µL) Available	Customizable 9 Analytes (50X concentration, 90 µL) Cat. No.	9-Plex Magnetic Premixed Beads
Anti-Human IFNε Bead	20	✔	HIFNE-MAG	✔
Anti-Human IFNα-2 Bead	25	✔	HINFA2-MAG	✔
Anti-Human IFNɣ Bead	26	✔	HIFNY-MAG	✔
Anti-Human IFNλ3 Bead	28	✔	HIFNL3-MAG	✔
Anti-Human IFNβ Bead	38	✔	HIFNB-MAG	✔
Anti-Human IFNω Bead	39	✔	HIFN0-MAG	✔
Anti-Human IFNɣ Receptor 1 Bead	43	✔	HIFNGR1-MAG	✔
Anti-Human IFNλ2 Bead	46	✔	HIFNL2-MAG	✔
Anti-Human IFNλ1 Bead	48	✔	HIFNL1-MAG	✔

Materials Required (Not included)

Reagents

MAGPIX® Drive Fluid PLUS (Cat. No. 40-50030), xMAP® Sheath Fluid PLUS (Cat. No. 40-50021), or xMAP® Sheath Concentrate PLUS (Cat. No. 40-50023)

Instrumentation/Materials

- Adjustable pipettes with tips capable of delivering 25 µL to 1000 µL
- Multichannel pipettes capable of delivering 5 µL to 50 µL, or 25 µL to 200 µL
- Reagent reservoirs
- Polypropylene microfuge tubes
- Rubber bands
- Aluminum foil
- Absorbent pads
- Laboratory vortex mixer
- Sonicator (Branson Ultrasonic Cleaner Model B200 or equivalent)
- Titer plate shaker (VWR® Microplate Shaker Cat. No. 12620-926 or equivalent)
- Luminex® 200™, HTS, FLEXMAP 3D®, MAGPIX® instrument with xPONENT® software, or xMAP® INTELLIFLEX instrument with INTELLIFLEX software by Luminex® Corporation
- Automatic plate washer for magnetic beads (BioTek® 405 LS and 405 TS, Cat. No. 40-094, 40-095, 40-096, 40-097 or equivalent) or Handheld Magnetic Separation Block (Cat. No. 40-285 or equivalent)

  Note: If a plate washer or handheld magnetic separation block for magnetic beads is not available, one can use a microtiter filter plate (Cat. No. MX-PLATE) to run the assay using a vacuum filtration unit (Vacuum Manifold, Cat. No. MSVMHTS00 or equivalent with Vacuum Pump, Cat. No. WP6111560 or equivalent).

Safety Precautions

- All blood components and biological materials should be handled as potentially hazardous. Follow universal precautions as established by the Centers for Disease Control and Prevention and by the Occupational Safety and Health Administration when handling and disposing of infectious agents.
- Sodium azide or ProClin™ has been added to some reagents as a preservative. Although the concentrations are low, Sodium azide and ProClin™ may react with lead and copper plumbing to form highly explosive metal azides. Dispose of unused contents and waste in accordance with international, federal, state, and local regulations.

Symbol Definitions

Ingredient	Cat. No.	Label	
10X Wash Buffer	L-WB		**Warning.** May cause an allergic skin reaction. Wear protective gloves. IF ON SKIN: Wash with plenty of soap and water.
Human Interferon Panel Standard & Human Interferon Panel QC1 & 2	HIFN-8130 & HIFN-6130		**Danger**. .Harmful if swallowed or if inhaled. Toxic in contact with skin. Causes serious eye damage. May cause damage to organs Respiratory Tract through prolonged or repeated exposure. May cause damage to organs Brain through prolonged or repeated exposure if swallowed. Harmful to aquatic life with long lasting effects. Do not breathe dust. Wash skin thoroughly after handling. Do not eat, drink or smoke when using this product. Use only outdoors or in a well-ventilated area. Avoid release to the environment. Wear protective gloves/ protective clothing/ eye protection/ face protection. IF SWALLOWED: Call a POISON CENTER/ doctor if you feel unwell. Rinse mouth. IF ON SKIN: Wash with plenty of water. Call a POISON CENTER/ doctor if you feel unwell. IF INHALED: Remove person to fresh air and keep comfortable for breathing. Call a POISON CENTER/ doctor if you feel unwell. IF IN EYES: Rinse cautiously with water for several minutes. Remove contact lenses, if present and easy to do. Continue rinsing .Get medical advice/ attention if you feel unwell. Take off contaminated clothing and wash before reuse. Store locked up. Dispose of contents/ container to an approved waste disposal plant.

Ingredient	Cat. No.	Label	
Serum Matrix	MXHSM-HIFN		**Danger.** Harmful if swallowed or if inhaled. Toxic in contact with skin. May cause damage to organs Brain through prolonged or repeated exposure if swallowed. Toxic to aquatic life with long lasting effects. Do not breathe dust. Wash skin thoroughly after handling. Do not eat, drink or smoke when using this product. Use only outdoors or in a well-ventilated area. Avoid release to the environment. Wear protective gloves/ protective clothing. IF SWALLOWED: Call a POISON CENTER/ doctor if you feel unwell. Rinse mouth. IF ON SKIN: Wash with plenty of water. Call a POISON CENTER/ doctor if you feel unwell. IF INHALED: Remove person to fresh air and keep comfortable for breathing. Call a POISON CENTER/ doctor if you feel unwell. Get medical advice/ attention if you feel unwell. Take off contaminated clothing and wash before reuse. Collect spillage. Store locked up. Dispose of contents/ container to an approved waste disposal plant.
Human Interferon Panel Detection Antibodies	HIFN-1130		**Warning.** Causes serious eye irritation. May cause damage to organs Respiratory Tract through prolonged or repeated exposure. Do not breathe mist or vapours. Wash skin thoroughly after handling. Wear eye protection/ face protection. IF IN EYES: Rinse cautiously with water for several minutes. Remove contact lenses, if present and easy to do. Continue rinsing. Get medical advice/ attention if you feel unwell. If eye irritation persists: Get medical advice/ attention. Dispose of contents/ container to an approved waste disposal plant.

Ingredient	Cat. No.	Label	
Streptavidin-Phycoerythrin	L-SAPE4		**Warning.** Causes serious eye irritation. IF IN EYES: Rinse cautiously with water for several minutes. Remove contact lenses, if present and easy to do. Continue rinsing.

Technical Guidelines

To obtain reliable and reproducible results, the operator should carefully read this entire manual and fully understand all aspects of each assay step before running the assay. The following notes should be reviewed and understood before the assay is set up.

- FOR RESEARCH USE ONLY. NOT FOR USE IN DIAGNOSTIC PROCEDURES.
- Do not use beyond the expiration date on the label.
- Do not mix or substitute reagents with those from other lots or sources.
- The Antibody-Immobilized Beads are light sensitive and must be protected from light at all times. Cover the assay plate containing beads with opaque plate lid or aluminum foil during all incubation steps.
- It is important to allow all reagents to warm to room temperature (20-25 °C) before use in the assay.
- Incomplete washing can adversely affect the assay outcome. All washing must be performed with the Wash Buffer provided.
- The standards prepared by serial dilution must be used within 1 hour of preparation. Discard any unused standards except the standard stock which may be stored at ≤ –20 °C for 1 month and at ≤ –80 °C for greater than one month.
- If samples fall outside the dynamic range of the assay, further dilute the samples with the appropriate Serum Matrix diluent and repeat the assay.
- Any unused mixed Antibody-Immobilized Beads may be stored in the Mixing Bottle at 2-8 °C for up to one month**.**
- During the preparation of the standard curve, make certain to mix the higher concentration well before making the next dilution. Use a new tip with each dilution.
- The plate should be read immediately after the assay is finished. If, however, the plate cannot be read immediately, seal the plate, cover with aluminum foil or an opaque lid, and store the plate at 2-8 °C for up to 24 hours. Prior to reading, agitate the plate on the plate shaker at room temperature for 10 minutes. Delay in reading a plate may result in decreased sensitivity for some analytes.
- The titer plate shaker should be set at a speed to provide maximum orbital mixing without splashing of liquid outside the wells. For the recommended plate shaker, this would be a setting of 5-7 which is approximately 500–800 rpm.

- Ensure that the needle probe is clean. This may be achieved by sonication and/or alcohol flushes.
- When reading the assay on the Luminex® 200™ instrument, adjust probe height according to the protocols recommended by Luminex® to the kit solid plate or to the recommended filter plates using 3 alignment discs. When reading the assay on the MAGPIX® instrument, adjust probe height according to the protocols recommended by Luminex® to the kit solid plate or to the recommended filter plates using 2 alignment discs. When reading the assay on the FLEXMAP 3D® instrument, adjust probe height according to the protocols recommended by Luminex® to the kit solid plate using 1 alignment disc.
- For the FLEXMAP 3D® instrument, when using the solid plate in the kit, the final resuspension should be with 150 µL Sheath Fluid PLUS in each well and 75 µL should be aspirated.
- For the xMAP® INTELLIFLEX instrument, adjust probe height based on the type of plate you are using, place an alignment disk or an alignment sphere in the well according to the protocol recommended by Luminex®.
- For cell culture supernatants or tissue extraction, use the culture or extraction medium as the matrix solution in background, standard curve and control wells. If samples are diluted in Assay Buffer, use the Assay Buffer as matrix.
- For serum/plasma samples that require further dilution beyond "1:2 dilution", use the Serum Matrix provided in the kit.
- For cell/tissue homogenate, the final cell or tissue homogenate should be prepared in a buffer that has a neutral pH, contains minimal detergents or strong denaturing detergents, and has an ionic strength close to physiological concentration. Avoid debris, lipids, and cell/tissue aggregates. Centrifuge samples before use.
- Vortex all reagents well before adding to plate.
- Some analytes are temperature sensitive. Please use freshly thawed samples.
- Use the **Hydration Buffer** provided in the kit for hydration of the Standard, Quality Controls, and Serum Matrix.

Sample Collection and Storage

Preparation of Serum Samples

- Allow the blood to clot for at least 30 minutes before centrifugation for 10 minutes at 1000 x *g*. Remove serum and assay immediately or aliquot and store samples at ≤ –20 °C.
- Avoid multiple (> 2) freeze/thaw cycles.
- When using frozen samples, it is recommended to thaw the samples completely, mix well by vortexing gently and centrifuge prior to use in the assay to remove particulates.
- 1:2 dilution Serum samples are used. (For example, 35 uL of sample and 35 µL of Assay Buffer) When further dilution is required, use Serum Matrix as the diluent.

Preparation of Plasma Samples

- Plasma collection using EDTA as an anti-coagulant is recommended. Centrifuge for 10 minutes at 1000 x *g* within 30 minutes of blood collection. Remove plasma and assay immediately or aliquot and store samples at ≤ –20 °C.
- Avoid multiple (> 2) freeze/thaw cycles.
- When using frozen samples, it is recommended to thaw the samples completely, mix well by vortexing gently and centrifuge prior to use in the assay to remove particulates.
- 1:2 dilution Plasma samples are used. (For example, 35 µL of sample and 35 µL of Assay Buffer) When further dilution is required, use Serum Matrix as the diluent.

Preparation of Tissue Culture Supernatant

- Centrifuge the sample to remove debris and assay immediately or aliquot and store samples at ≤ –20 °C.
- Avoid multiple (> 2) freeze/thaw cycles.
- Tissue culture supernatant may require a dilution with an appropriate control medium prior to assay. Tissue/cell extracts should be done in neutral buffers containing reagents and conditions that do not interfere with assay performance. Excess concentrations of detergent, salt, denaturants, high or low pH, etc. will negatively affect the assay. Organic solvents should be avoided. The tissue/cell extract samples should be free of particles such as cells or tissue debris.

NOTE:

- A maximum of 25 µL per well of 1:2 diluted sample can be used.
- All samples must be stored in polypropylene tubes. **DO NOT STORE SAMPLES IN GLASS.**
- Avoid debris, lipids and cells when using samples with gross hemolysis or lipemia.
- Care must be taken when using heparin as an anti-coagulant since an excess of heparin will provide falsely high values. Use no more than 10 IU heparin per mL of blood collected.

Preparation of Reagents for Immunoassay

Preparation of Antibody-Immobilized Beads

- If premixed beads are used, sonicate the premixed bead bottle 30 seconds and then vortex for 1 minute before use.
- For individual vials of beads, sonicate each antibody-bead vial for 30 seconds; vortex for 1 minute. Add 60 µL from each antibody-bead vial to the Mixing Bottle and bring final volume to 3.0 mL with Bead Diluent. Vortex the mixed beads well. Unused portion may be stored at 2-8 °C for up to one month.

  Note: Due to the composition of magnetic beads, you may notice a slight color in the bead solution. This does not affect the performance of the beads or the kit.

  Example: When using 9 antibody-immobilized beads, add 60 µL from each of the 9 bead vials to the Mixing Bottle. Then add 2.46 mL Bead Diluent.

Preparation of Quality Controls

Before use, reconstitute Quality Control 1 and Quality Control 2 with 250 µL **Hydration Buffer**. Invert the vial several times to mix and vortex. Allow the vial to sit for 5-10 minutes. Transfer the reconstituted Quality Control 1 and Quality Control 2 into two polypropylene microfuge tubes and set in an ice bath. These should be added to the plate within 1 hour of reconstitution. Unused portion may be stored at ≤ −20 °C for up to one month.

Preparation of Wash Buffer

Bring the 10X Wash Buffer to room temperature and mix to bring all salts into solution. Dilute 60 mL of 10X Wash Buffer with 540 mL deionized water. Store the unused portion at 2-8 °C for up to one month.

Preparation of Serum Matrix

This step is required for serum or plasma samples only.

Add 1 mL **Hydration Buffer** to the bottle containing lyophilized Serum Matrix. Mix well. Allow at least 10 minutes for complete reconstitution. Add 1 mL of Assay Buffer. Mix well. Leftover reconstituted Serum Matrix should be stored at ≤ −20 °C for up to one month.

Preparation of Human Interferon Panel Standard

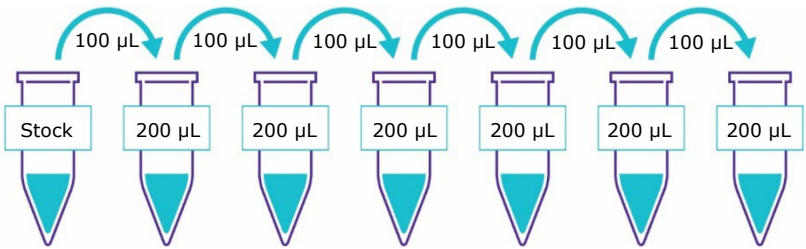
1. Prior to use, reconstitute the Human Interferon Standard with 250 µL **Hydration Buffer**. Refer to table below for analyte concentrations. Invert the vial several times to mix. Vortex the vial for 10 seconds. Allow the vial to sit for 5-10 minutes. Transfer the reconstituted standard to a polypropylene microfuge tube. This will be used as "Standard 7"; This reconstituted standard and the serially diluted standards in the following steps should be set in an ice bath, during the assay procedure. These need to be added to the plate within 1 hour of preparation. The unused portion of the "Standard 7" may be stored at ≤ −20 °C for up to one month.

2. Preparation of Working Standards
   Label 6 polypropylene microfuge tubes Standard 1 through Standard 6. Add 100 µL of Assay Buffer to each of the 6 tubes. Prepare serial dilutions by adding 50 µL of the reconstituted standard to the Standard 6 tube, mix well and transfer 50 µL of Standard 6 to the Standard 5 tube, mix well and transfer 50 µL of Standard 5 to the Standard 4 tube, mix well and transfer 50 µL of Standard 4 to the Standard 3 tube, mix well and transfer 50 µL of Standard 3 to the Standard 2 tube, mix well and transfer 50 µL of Standard 2 to the Standard 1 tube and mix well. The 0 pg/mL standard (Background) will be Assay Buffer.

Standard No.	Add Hydration Buffer	Add Standard (volume)
Standard 7	250 µL	0

Standard No.	Add Assay Buffer	Add Standard (volume)
Standard 6	200 µL	100 µL of Standard 7
Standard 5	200 µL	100 µL of Standard 6
Standard 4	200 µL	100 µL of Standard 5
Standard 3	200 µL	100 µL of Standard 4
Standard 2	200 µL	100 µL of Standard 3
Standard 1	200 µL	100 µL of Standard 2

Preparation of Standards



Reconstituted Standard 7 | Standard 6 | Standard 5 | Standard 4 | Standard 3 | Standard 2 | Standard 1

Standard	**IFNε** (pg/mL)	**IFNα2** (pg/mL)	**IFNɣ** (pg/mL)
Standard 1	274	34	4.1
Standard 2	823	103	12
Standard 3	2,469	309	37
Standard 4	7,407	926	111
Standard 5	22,222	2,778	333
Standard 6	66,667	8,333	1,000
Standard 7	200,000	25,000	3,000

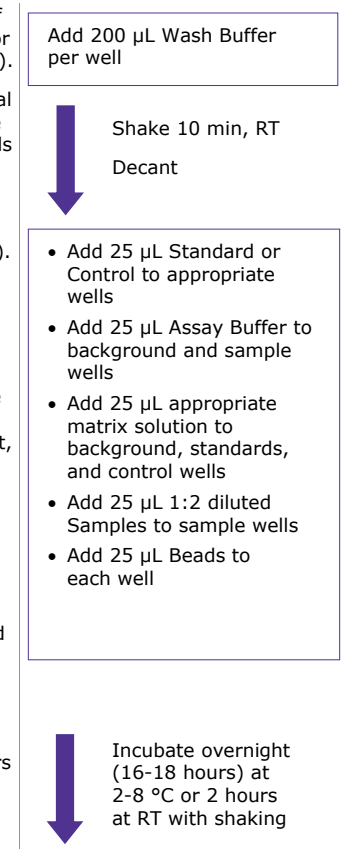
Standard	**IFNλ3** (pg/mL)	**IFNβ** (pg/mL)	**IFNω** (pg/mL)
Standard 1	11	3.4	14
Standard 2	33	10	41
Standard 3	99	31	124
Standard 4	296	93	370
Standard 5	889	278	1,111
Standard 6	2,667	833	3,333
Standard 7	8,000	2,500	10,000

Standard	**IFNγR1** (pg/mL)	**IFNλ2** (pg/mL)	**IFNλ1** (pg/mL)
Standard 1	14	25	4.8
Standard 2	41	74	14
Standard 3	124	222	43
Standard 4	370	667	130
Standard 5	1,111	2,000	389
Standard 6	3,333	6,000	1,167
Standard 7	10,000	18,000	3,500

Immunoassay Procedure

- Prior to beginning this assay, it is imperative to read this protocol completely and to thoroughly understand the Technical Guidelines.
- Allow all reagents to warm to room temperature (20-25 °C) before use in the assay.
- Diagram the placement of Standards 0 (Background), Standard 1 through 7, Controls 1 and 2, and Samples on Well Map Worksheet in a vertical configuration. **Note:** Most instruments will only read the 96-well plate vertically by default. It is recommended to run the assay in duplicate.

3. Add 200 µL of Wash Buffer into each well of the plate. Seal and mix on a plate shaker for 10 minutes at room temperature (20-25 °C).
4. Decant Wash Buffer and remove the residual amount from all wells by inverting the plate and tapping it smartly onto absorbent towels several times.
5. Add 25 µL of each Standard or Control into the appropriate wells. Assay Buffer should be used for 0 pg/mL standard (Background).
6. Add 25 µL of Assay Buffer to the sample wells.
7. Add 25 µL of appropriate matrix solution to the background, standards, and control wells. When assaying serum or plasma, use the Serum Matrix provided in the kit. When assaying tissue culture or other supernatant, use proper control culture medium as the matrix solution.
8. Add 25 µL of Sample (1:2 diluted serum or plasma samples) into the appropriate wells.
9. Vortex Mixing Bottle and add 25 µL of the Mixed or Premixed Beads to each well. **Note:** During addition of Beads, shake bead bottle intermittently to avoid settling.
10. Seal the plate with a plate sealer. Wrap the plate with foil and incubate with agitation on a plate shaker overnight (16-18 hours) at 2-8 °C. Alternatively, incubate for 2 hours at room temperature (20-25 °C).



11. Gently remove well contents and wash plate 3 times following instructions listed in the Plate Washing section.
12. Add 25 µL of Detection Antibodies into each well.
Note: Allow the Detection Antibodies to warm to room temperature prior to addition.
13. Seal, cover with foil and incubate with agitation on a plate shaker for 1 hour at room temperature (20-25 °C). **DO NOT ASPIRATE AFTER INCUBATION.**
14. Add 25 µL Streptavidin-Phycoerythrin to each well containing the 25 µL of Detection Antibodies.
15. Seal, cover with foil and incubate with agitation on a plate shaker for 30 minutes at room temperature (20-25 °C).
16. Gently remove well contents and wash plate 3 times following instructions listed in the Plate Washing section.
17. Add 150 µL of Sheath Fluid PLUS (or Drive Fluid PLUS if using MAGPIX®) to all wells. Resuspend the beads on a plate shaker for 5 minutes.
18. Run plate on Luminex® 200™, HTS, FLEXMAP 3D®, MAGPIX® instrument with xPONENT® software or xMAP® INTELLIFLEX instrument with INTELLIFLEX software.
19. Save and analyze the Median Fluorescent Intensity (MFI) data using a 5-parameter logistic or spline curve-fitting method for calculating analyte concentrations in samples. **Note:** For diluted samples, final sample concentrations should be multiplied by the dilution factor.

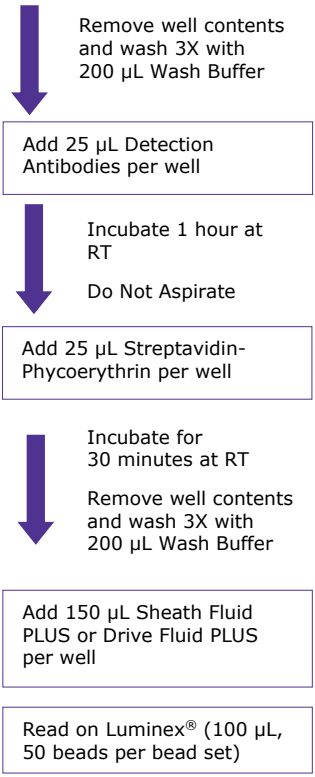


Plate Washing

If using a solid plate, use either a handheld magnet or magnetic plate washer.

- Handheld magnet (Cat. No. 40-285)
  Rest plate on magnet for 60 seconds to allow complete settling of magnetic beads. Remove well contents by gently decanting the plate in an appropriate waste receptacle and gently tapping on absorbent pads to remove residual liquid. Wash plate with 200 µL of Wash Buffer by removing plate from magnet, adding Wash Buffer, shaking for 30 seconds, reattaching to magnet, letting beads settle for 60 seconds and removing well contents as previously described after each wash. Repeat wash steps as recommended in
  Assay Procedure.
- Magnetic plate washer (Cat. No. 40-094, 40-095, 40-096 and 40-097)
  Please refer to specific automatic plate washer manual for appropriate equipment settings. Please note that after the final aspiration, there will be approximately 25 µL of residual wash buffer in each well. This is expected when using the BioTek® plate washer and this volume does not need to be aspirated from the plate.

  If using an automatic plate washer other than BioTek® 405 LS or 405 TS, please refer to the manufacturer's recommendations for programming instructions.

Equipment Settings

Luminex® 200™, HTS, FLEXMAP 3D®, MAGPIX® instruments with xPONENT® software and xMAP® INTELLIFLEX instrument with INTELLIFLEX software:

These specifications are for the above listed instruments and software. Luminex® instruments with other software (for example, MasterPlex®, StarStation, LiquiChip, Bio-Plex® Manager™, LABScan™100) would need to follow instrument instructions for gate settings and additional specifications from the vendors for reading Luminex® magnetic beads.

For magnetic bead assays, each instrument must be calibrated, and performance verified with the indicated calibration and verification kits.

Instrument	Calibration Kit	Verification Kit
Luminex® 200™ and HTS	xPONENT® 3.1 compatible Calibration Kit (Cat. No. LX2R-CAL-K25)	Performance Verification Kit (Cat. No. LX2R-PVER-K25)
FLEXMAP 3D®	FLEXMAP 3D® Calibrator Kit (Cat. No. F3D-CAL-K25)	FLEXMAP 3D® Performance Verification Kit (Cat. No. F3D-PVER-K25)
xMAP® INTELLIFLEX	xMAP® INTELLIFLEX Calibration Kit (Cat. No. IFX-CAL-K20)	xMAP® INTELLIFLEX Performance Verification Kit (Cat. No. IFX-PVER-K20)
MAGPIX®	MAGPIX® Calibration Kit (Cat. No. MPX-CAL-K25)	MAGPIX® Performance Verification Kit (Cat. No. MPX-PVER-K25)

Note: When setting up a Protocol using the xPONENT® software, you must select MagPlex® as the Bead Type in the Acquisition settings.

Note: These assays cannot be run on any instruments using Luminex® IS 2.3 or Luminex® 1.7 software.

The Luminex® probe height must be adjusted to the plate provided in the kit. Please use Cat. No. MAG-PLATE, if additional plates are required for this purpose.

Events	50, per bead
Sample Size	100 µL
Gate Settings	8,000 to 15,000
Reporter Gain	Default (low PMT)
Time Out	60 seconds
Bead Set	Customizable 9-plex Beads

IFNε	20
IFNα-2	25
IFNɣ	26
IFNλ3	28
IFNβ	38
IFNω	39
IFNɣ R1	43
IFNλ2	46
IFNλ1	48

Quality Controls

The ranges for each analyte in Quality Control 1 and 2 are provided on the card insert or can be located at our website SigmaAldrich.com using the catalogue number as the keyword.

Assay Characteristics

Cross-Reactivity

There was no or negligible cross-reactivity between the antibodies for an analyte and any of the other analytes in this panel.

Assay Sensitivities

(minimum detectable concentrations, pg/mL)

Minimum Detectable Concentration (MinDC) is calculated using MILLIPLEX® Analyst 5.1. It measures the true limits of detection for an assay by mathematically determining what the empirical MinDC would be if an infinite number of standard concentrations were run for the assay under the same conditions.

	Overnight Protocol (n = 8 Assays)		2 Hour Protocol (n = 2 Assays)	
Analyte	**MinDC (pg/mL)**	**MinDC+2SD (pg/mL)**	**MinDC (pg/mL)**	**MinDC+2SD (pg/mL)**
IFNε	100.20	259.28	245.14	253.80
IFNα-2	21.43	42.90	10.21	23.31
IFNγ	1.27	1.71	0.57	0.78
IFNλ3	2.77	4.88	2.27	2.44
IFNβ	1.56	3.34	1.41	3.84
IFNω	3.57	7.99	4.01	7.94
IFNγ R1	3.53	5.93	0.32	0.97
IFNλ2	10.14	14.02	12.35	15.69
IFNλ1	2.79	4.63	3.51	4.16

Precision

Intra-assay precision is generated from the mean of the %CVs from 16 reportable results across two different concentrations of analytes in a single assay. Inter-assay precision is generated from the mean of the %CVs across two different concentrations of analytes across 10 different assays.

	Overnight Protocol		2 Hour Protocol
Analyte	**Intra-assay %CV**	**Inter-assay %CV**	**Intra-assay %CV**
IFNε	<10	<20	<10
IFNα-2	<10	<20	<10
IFNɣ	<10	<20	<10
IFNλ3	<10	<20	<10
IFNβ	<10	<20	<10
IFNω	<10	<20	<10
IFNɣ R1	<10	<20	<10
IFNλ2	<10	<20	<10
IFNλ1	<10	<20	<10

Accuracy

Spike Recovery: The data represent mean percent recovery of spiked standards ranging from low, medium, and high concentration in serum matrices (n=6).

	Overnight Protocol	2 Hour Protocol
Analyte	**% Recovery in Serum Matrix**	**% Recovery in Serum Matrix**
IFNε	94	101
IFNα-2	89	95
IFNɣ	96	98
IFNλ3	98	98
IFNβ	96	99
IFNω	96	99
IFNɣ R1	97	102
IFNλ2	94	101
IFNλ1	96	101

Troubleshooting

Problem	Probable Cause	Solution
Insufficient bead count	Plate washer aspirate height set too low	Adjust aspiration height according to manufacturers' instructions.
	Bead mix prepared inappropriately	Sonicate bead vials and vortex just prior to adding to bead mix bottle according to protocol. Agitate bead mix intermittently in reservoir while pipetting this into the plate.
	Samples cause interference due to particulate matter or viscosity	See above. Also sample probe may need to be cleaned with alcohol flushes, back flushes and washes; or if needed, probe should be removed and sonicated.
	Probe height not adjusted correctly	When reading the assay on the Luminex® 200™ instrument, adjust probe height to the kit solid plate using 3 alignment discs. When reading the assay on the MAGPIX® instrument, adjust probe height to the kit solid plate using 2 alignment discs. When reading the assay on the FLEXMAP 3D® instrument, adjust probe height to the kit solid plate using 1 alignment disc. When reading the assay on the xMAP® INTELLIFLEX instrument, adjust probe height based on the type of plate you are using, place an alignment disk or an alignment sphere in the well according to the protocol recommended by Luminex®.
Background is too high	Background wells were contaminated	Avoid cross-well contamination by using sealer appropriately and pipetting with multichannel pipettes without touching reagent in plate.
	Matrix used has endogenous analyte or interference	Check matrix ingredients for cross-reacting components (for example, interleukin modified tissue culture medium).
	Insufficient washes	Increase number of washes.

Problem	Probable Cause	Solution
Beads not in region or gate	Luminex® instrument not calibrated correctly or recently	Calibrate Luminex® instrument based on manufacturer's instructions, at least once a week or if temperature has changed by > 3 °C.
	Gate settings not adjusted correctly	Some Luminex® instruments (for example, Bio-Plex®) require different gate settings than those described in the kit protocol. Use instrument default settings.
	Wrong bead regions in protocol template	Check kit protocol for correct bead regions or analyte selection.
	Incorrect sample type used	Samples containing organic solvents or if highly viscous should be diluted or dialyzed as required.
	Instrument not washed or primed	Prime the Luminex® instrument 4 times to rid it of air bubbles, wash 4 times with sheath fluid or water if there is any remnant alcohol or sanitizing liquid.
	Beads were exposed to light	Keep plate and bead mix covered with dark lid or aluminum foil during all incubation steps.
Signal for whole plate is same as background	Incorrect or no Detection Antibody was added	Add appropriate Detection Antibody and continue.
	Streptavidin-Phycoerythrin was not added	Add Streptavidin-Phycoerythrin according to protocol. If Detection Antibody has already been removed, sensitivity may be low.
Low signal for standard curve	Detection Antibody may have been removed prior to adding Streptavidin-Phycoerythrin	May need to repeat assay if desired sensitivity not achieved.
	Incubations done at inappropriate temperatures, timings or agitation	Assay conditions need to be checked.

Problem	Probable Cause	Solution
Signals too high, standard curves are saturated	Calibration target value set too high	With some Luminex® instruments (for example, Bio-Plex®) default target setting for RP1 calibrator is set at high PMT. Use low target value for calibration and reanalyze plate.
	Plate incubation was too long with standard curve and samples	Use shorter incubation time.
Sample readings are out of range	Samples contain no or below detectable levels of analyte	If below detectable levels, it may be possible to use higher sample volume. Check with technical support for appropriate protocol modifications.
	Samples contain analyte concentrations higher than highest standard point	Samples may require dilution and reanalysis for just that particular analyte.
	Standard curve was saturated at higher end of curve	See above.
High variation in samples and/or standards	Multichannel pipette may not be calibrated	Calibrate pipettes.
	Plate washing was not uniform	Confirm all reagents are removed completely in all wash steps.
	Samples may have high particulate matter or other interfering substances	See above.
	Plate agitation was insufficient	Plate should be agitated during all incubation steps using an orbital plate shaker at a speed where beads are in constant motion without causing splashing.
	Cross-well contamination	Check when reusing plate sealer that no reagent has touched sealer. Care should be taken when using same pipette tips that are used for reagent additions and that pipette tip does not touch reagent in plate.

Product Ordering

Order products online at SigmaAldrich.com.

Reagents

Description	Cat. No.
Human Interferon Panel Standard	HIFN-8130
Human Interferon Panel Quality Controls 1 and 2	HIFN-6130
Serum Matrix	MXHSM-HIFN
Human Interferon Panel Detection Antibodies	HIFN-1130
Streptavidin-Phycoerythrin	L-SAPE4
Assay Buffer	L-AB
Set of two 96-Well plates with sealers	MAG-PLATE
10X Wash Buffer	L-WB
Bead Diluent	LBD
Human Interferon Panel 9-Plex Premixed Beads*	HIFNPMX9-MAG
Hydration Buffer	L-HB

* For individual beads, see page 25.

Antibody-Immobilized Magnetic Beads

Analyte	Bead No.	Cat. No.
IFNε	20	HIFNE-MAG
IFNα-2	25	HINFA2-MAG
IFNɣ	26	HIFNY-MAG
IFNλ3	28	HIFNL3-MAG
IFNβ	38	HIFNB-MAG
IFNω	39	HIFN0-MAG
IFNɣ R1	43	HIFNGR1-MAG
IFNλ2	46	HIFNL2-MAG
IFNλ1	48	HIFNL1-MAG

Well Map

	1	2	3	4	5	6	7	8	9	10	11	12
A	Standard 0 (Background)	Standard 4	QC-1 Control	Etc.								
B	Standard 0 (Background)	Standard 4	QC-1 Control									
C	Standard 1	Standard 5	QC-2 Control									
D	Standard 1	Standard 5	QC-2 Control									
E	Standard 2	Standard 6	Sample 1									
F	Standard 2	Standard 6	Sample 1									
G	Standard 3	Standard 7	Sample 2									
H	Standard 3	Standard 7	Sample 2									

Notice

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Contact Information

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Technical Assistance

Visit the tech service page at SigmaAldrich.com/techservice.

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Safety Data Sheets (SDS)

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00006883 Rev 02/25