User Guide

MILLIPLEX® Human IL-18 Singleplex Magnetic Bead Kit

96-Well Plate Assay

HIL18MAG-66K

Introduction

Interleukin-18 (IL-18, also known as interferon-gamma inducing factor) is a member of the IL-1 cytokine superfamily and acts as a pleiotropic cytokine that modulates both innate and acquired immune responses. IL-18 is first produced as an inactive intracellular precursor lacking a secretion signal peptide. To be activated, the IL-18 precursor is cleaved by cysteine protease caspase-1 to generate the mature form which can be released from the cell. Binding of activated IL-18 to the IL-18 receptor alpha chain (IL-18Rα) and IL-18 beta chain co-receptor (IL-18Rβ) leads to a cascade of reactions causing the activation of NF-κB transcription factor, which induces the production of proinflammatory cytokines. IL-18 augments natural killer cell activity in spleen cells and induces interferon gamma production in CD4 T-helper (Th) type I lymphocytes. In addition, it is also involved in regulating the activity of other immune cell types such as Th2, Th17, CD8 T cells and neutrophils. In healthy humans and animals, the IL-18 precursor is constitutively present in nearly all cells including both hematopoietic and nonhematopoietic cells, such as blood monocytes and the gastrointestinal epithelial cells, respectively. The activity of IL-18 is balanced by its natural inhibitory protein IL-18 binding protein (IL-18BP) and depends on the level of IL-18 receptors (IL-18R) on the surface of the responding cells. Disrupted regulation of IL-18 bioactivity is linked to multiple types of diseases including chronic inflammatory diseases, autoimmune diseases, a variety of cancers, and infectious diseases. It also has been implicated to play a role in emphysema, myocardial function, cardiovascular disease, metabolic syndrome, acute kidney injury and neurodegeneration.

The MILLIPLEX® portfolio offers the broadest selection of analytes across a wide range of disease states and species. Once the analyte or 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: 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 cocktail (or cocktails for multiplex kits) designed to yield consistent analyte profiles within panel

In addition, each panel and kit meets stringent manufacturing criteria to ensure batch-to-batch reproducibility. The MILLIPLEX® Human IL-18 Singleplex Magnetic Bead Kit enables you to focus on the therapeutic potential of cytokines as well as 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.

The MILLIPLEX® Human IL-18 Singleplex Magnetic Bead Kit is part of the most versatile system available for cytokine and chemokine 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.

The MILLIPLEX® Human IL-18 Singleplex Magnetic Bead Kit is a singleplex kit to be used for the quantification of IL-18 in serum, plasma, and tissue/cell supernatant samples. Additionally, MILLIPLEX® Human IL-18 Singleplex Magnetic Bead Kit can be combined and assayed within the following kits:

- MILLIPLEX® Human Cytokine/Chemokine Magnetic Bead Panel II Cat. No. HCYP2MAG-62K (with the exception of the CTACK/CCL27 assay, see below for further information)
- MILLIPLEX® Human Th17 Magnetic Bead Panel Cat. No. HTH17MAG-14K (or HT17MG-14K-PX25, or the corresponding bulk format kit)

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 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 IL-18 Standard (25X)	Lyophilized	1 vial	**HIL18-8066
Human IL-18 Quality Controls 1 and 2 (25X)	Lyophilized	1 vial each	HIL18-6066
*Serum Matrix	Lyophilized	1 bottle	*HIL18-SM
Bead Diluent	3.5 mL	1 bottle	LBD
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 IL-18 Detection Antibodies (20X)	200 µL	1 vial	HIL18-1066
*Streptavidin-Phycoerythrin	5.5 mL	1 bottle	*L-SAPE5
Mixing Bottle	-	1 bottle	-

*If combining Human IL-18 into another kit, use the corresponding reagents provided in the other kit.

**The MILLIPLEX® Human IL-18 Magnetic Bead Panel Standard is calibrated against the International Standards for IL-18 (WHO NIBSC 03/200).

Human IL-18 Singleplex Antibody-Immobilized Magnetic Beads

Bead/Analyte Name	Luminex® Magnetic Bead Region	Cat. No.	Volume	Concentration in vial
Anti-Human IL-18 Bead	62	HIL18-MAG	90 µL	50X

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
- Polypropylene Conical Tube capable of accommodating > 3.2 mL
- Rubber Bands
- Aluminum Foil
- Absorbent Pads
- Laboratory Vortex Mixer
- Sonicator (Branson Ultrasonic Cleaner Model No. B200 or equivalent)
- Titer Plate Shaker (VWR® Microplate Shaker Cat. No. 12620-926 or equivalent)
- Luminex® 200™, HTS, FLEXMAP 3D®, MAGPIX® with xPONENT® software or xMAP® INTELLIFLEX 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 has been added to some reagents as a preservative. Although the concentrations are low, Sodium azide 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	
Human IL-18 Standard	HIL18-8066		**Danger.** Harmful if swallowed. Causes serious eye damage. Harmful to aquatic life with long lasting effects. Avoid release to the environment. Wear eye 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.
Human IL-18 Quality Control 1 & 2	HIL18-6066		**Danger.** Harmful if swallowed. Causes serious eye damage. Harmful to aquatic life with long lasting effects. Avoid release to the environment. Wear eye 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.
Human IL-18 Detection Antibody	HIL18-1066		**Warning.** Causes serious eye irritation. Harmful to aquatic life with long lasting effects. Avoid release to the environment. IF IN EYES: Rinse cautiously with water for several minutes. Remove contact lenses, if present and easy to do. Continue rinsing.
Serum Matrix	HIL18-SM	no symbol required	Harmful to aquatic life with long lasting effects. Avoid release to the environment.
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.
Streptavidin-Phycoerythrin	L-SAPE5		**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 25X concentrated 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 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 a dilution greater than "neat", 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.

Sample Collection and Storage

Note: If combining the Human IL-18 assay into another kit, follow that kit's "Sample Collection and Storage" procedure. If using the Human IL-18 assay as a singleplex kit, follow the instructions below.

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 and centrifuge prior to use in the assay to remove particulates.
- Neat serum samples are used. If 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 and centrifuge prior to use in the assay to remove particulates.
- Neat plasma samples are used. If 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 neat serum or plasma can be used. Tissue culture or other media may also 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: Single Human IL-18 Analyte Only

Note: If combining the Human IL-18 assay into another kit, disregard this section and follow the instructions in "Preparations of Reagents for Immunoassay: Human IL-18 with Other Kits". If using the Human IL-18 assay as a singleplex kit, follow the instructions below.

Preparation of Antibody-Immobilized Beads (HIL18-MAG)

Sonicate the HIL18-MAG antibody-bead vial for 30 seconds; vortex for 1 minute. Add 60 µL from antibody-bead vial to the Mixing Bottle and bring final volume to 3.0 mL using 2.94 mL of 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.)

Preparation of Human IL-18 Detection Antibody (HIL18-1066)

Prior to use, the 20X concentrated HIL-18 Detection Antibody must be diluted with Assay Buffer. Add 160 µL of HIL-18 Detection Antibody to a polypropylene conical tube that can accommodate > 3.2 mL. Bring final volume to 3.2 mL using 3.04 mL of Assay Buffer. Mix well prior to addition in assay.

Preparation of Human IL-18 Quality Controls (HIL18-6066)

Prior to use, reconstitute the lyophilized HIL-18 Quality Control 1 (QC1) and Quality Control 2 (QC2) with 200 µL deionized water to produce 25X concentrated HIL-18 Quality Controls. Invert the vials several times to mix then vortex. Allow the vials to sit for 5-10 minutes. Transfer the reconstituted Quality Control 1 and Quality Control 2 into two appropriately labeled polypropylene microfuge tubes. Label 2 polypropylene microfuge tubes QC1 and QC2 and add 240 µL of Assay Buffer and 10 µL of each 25X concentrated QC1 and QC2, respectively, to generate the 1X Quality Control working stocks. Vortex to mix. The 1X working stocks must be used immediately. The unused portions of the 25X Quality Controls may be stored at ≤ −20 °C for up to one month.

Preparation of Wash Buffer (L-WB)

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 (HIL18-SM)

This step is required for serum or plasma samples only.

Add 1.0 mL of deionized water to the bottle containing lyophilized Serum Matrix. Mix well. Allow at least 10 minutes for complete reconstitution. Leftover reconstituted Serum Matrix should be stored at ≤ –20 °C for up to one month.

Preparation of Human IL-18 Standard (HIL18-8066)

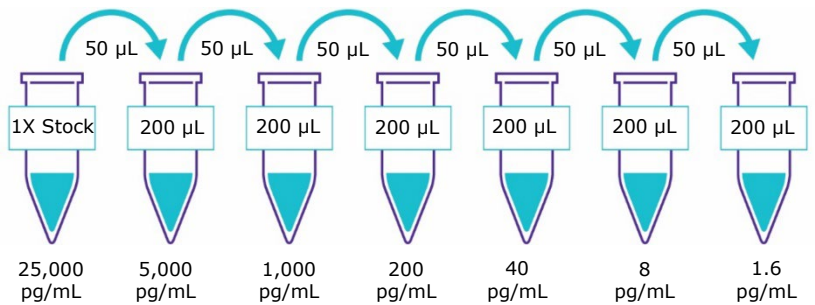
1. Prior to use, reconstitute the lyophilized HIL-18 Standard with 200 µL deionized water to produce the 25X concentrated stock at 625 ng/mL for HIL-18. Invert the vial several times to mix. Vortex the vial for 10 seconds. Allow the vial to sit for 5-10 minutes. Transfer the reconstituted standard to an appropriately labeled polypropylene microfuge tube. Label 1 polypropylene microfuge tube 25,000 pg/mL and add 240 µL of Assay Buffer and 10 µL of the 25X concentrated standard. Vortex to mix. This will be used as the 1X working stock "25,000 pg/mL Standard". The 1X working stock must be used immediately. The unused portion of the 25X HIL-18 Standard may be stored at ≤ –20 °C for up to one month.

2. Preparation of Working Standards
   Label 6 polypropylene microfuge tubes 5,000, 1,000, 200, 40, 8, and 1.6 pg/mL. Add 200 µL of Assay Buffer to each of the 6 tubes. Prepare serial dilutions by adding 50 µL of the 1X working stock 25,000 pg/mL standard to the 5,000 pg/mL tube, mix well and transfer 50 µL of the 5,000 pg/mL to the 1,000 pg/mL tube, mix well and transfer 50 µL of the 1,000 pg/mL standard to the 200 pg/mL standard tube, mix well and transfer 50 µL of the 200 pg/mL standard to the 40 pg/mL standard tube, mix well and transfer 50 µL of the 40 pg/mL standard to the 8 pg/mL standard tube, mix well and transfer 50 µL of the 8 pg/mL standard to the 1.6 pg/mL standard tube and mix well. The 0 pg/mL standard (Background) will be Assay Buffer.

Standard Concentration	Add Deionized Water (µL)	Add Standard (volume)
625,000 pg/mL	200	0

Standard Concentration (pg/mL)	Add Assay Buffer (µL)	Add Standard (volume)
25,000	240	10 µL of 625,000 pg/mL
5,000	200	50 µL of 25,000 pg/mL
1,000	200	50 µL of 5,000 pg/mL
200	200	50 µL of 1,000 pg/mL
40	200	50 µL of 200 pg/mL
8	200	50 µL of 40 pg/mL
1.6	200	50 µL of 8 pg/mL

Preparation of Standards



Preparation of Reagents for Immunoassay: Human IL-18 with Other Kits

Instructions for Combining the Human IL-18 Assay into Other Kits (or, "Combined" Kit)

Note: These instructions are meant to be followed in conjunction with the corresponding reagent preparation instructions outlined in the following combinable immunoassay kits:

- MILLIPLEX® Human Cytokine/Chemokine Magnetic Bead Panel II Cat. No. HCYP2MAG-62K (with the exception of the CTACK/CCL27 assay, see below for further information)
- MILLIPLEX® Human Th17 Magnetic Bead Panel Cat. No. HTH17MAG-14K (or HT17MG-14K-PX25, or the corresponding bulk format kit)

Preparation of Antibody-Immobilized Beads Combined with Human IL-18 Beads (HIL18-MAG)

Note: Due to bead region overlap, CTACK/CCL27 cannot be assayed with HIL-18.

- If premixed beads are used in the "COMBINED" kit, sonicate the HIL-18 bead vial 30 seconds and then vortex for 1 minute. Add 70 µL of the HIL-18 bead to the premixed bead bottle. Vortex for 1 minute prior to addition in assay.
- If individual vials of beads are used in the "COMBINED" kit, follow the corresponding instructions outlined in the kit protocol and incorporate the HIL-18 bead.

Preparation of Detection Antibody Combined with Human IL-18 Detection Antibody (HIL18-1066)

Add 160 µL of the 20X concentrated HIL-18 Detection Antibody to the 3.2 mL Detection Antibody Cocktail provided in the "COMBINED" kit. Mix well prior to addition in assay.

Preparation of Quality Controls Combined with Human IL-18 Quality Controls (HIL18-6066)

Prior to use, reconstitute the HIL-18 Quality Control 1 and Quality Control 2 with 200 µL deionized water. Invert the vial several times to mix then vortex. Allow the vial to sit for 5-10 minutes. Transfer the reconstituted Quality Control 1 and Quality Control 2 into two appropriately labeled polypropylene microfuge tubes.

Adding HIL-18 Quality Controls to HTH17MAG-14K

Label 2 polypropylene microfuge tubes QC1 and QC2. Add 288 µL of deionized water to each of the 2 tubes. Prepare final concentrations of HIL-18 Quality Control 1 and 2 by adding 12 µL of the reconstituted Quality Control 1 and 2 to the QC1 and QC2 tubes, respectively. Mix well and use immediately for the following reconstitution.

Reconstitute the Quality Controls 1 and 2 of the kit that Human IL-18 is being combined into with 250 µL of the HIL-18 QC1 and QC2, respectively. Invert the vials several times to mix and vortex. Allow the vials to sit for 5-10 minutes.

Adding HIL-18 Quality Controls to HCYP2MAG-62K

Label 2 polypropylene microfuge tubes QC1 and QC2. Add 1,240 µL of deionized water to each of the 2 tubes. Prepare final concentrations of HIL-18 Quality Control 1 and 2 by adding 10 µL of the reconstituted Quality Control 1 and 2 to the QC1 and QC2 tubes, respectively. Mix well and use immediately for the following reconstitution.

Reconstitute the Quality Controls 1 and 2 of the kit that Human IL-18 is being combined into with 250 µL of the 1X HIL-18 QC1 and QC2, respectively. Invert the vials several times to mix and vortex. Allow the vials to sit for 5-10 minutes. Transfer the reconstituted Quality Control 1 and Quality Control 2 into two appropriately labeled polypropylene microfuge tubes.

Preparation of Wash Buffer (L-WB)

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

Do not use HIL-18 Serum Matrix (HIL18-SM) if combining HIL-18 with another kit. Use the Serum Matrix provided in the "Combined" kit and follow corresponding instructions.

Preparation of Standard Combined with Human IL-18 Standard (HIL18-8066)

Prior to use, reconstitute the HIL-18 Standard with 200 µL deionized water to produce the concentrated stock at 625 ng/mL. 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 an appropriately labeled polypropylene microfuge tube.

Adding HIL-18 Standard to HTH17MAG-14K

In these two kits, the highest HIL-18 Standard concentration will be 25,000 pg/mL. Label a polypropylene microfuge tube 25,000 pg/mL and add 288 µL of deionized water. Prepare final concentration of HIL-18 Standard by adding 12 µL of the reconstituted 625 ng/mL Human IL-18 Standard to the 25,000 pg/mL tube. Mix well and use immediately for the following reconstitution.

Reconstitute the lyophilized standard of the kit that Human IL-18 is being combined into with 250 µL of the 25,000 pg/mL HIL-18 Standard. Invert the vial several times to mix and vortex. Allow the vial to sit for 5-10 minutes. Transfer the reconstituted standard to an appropriately labeled polypropylene microfuge tube. Continue with the standard serial dilution instructions as outlined in the HTH17MAG-14K kit protocol.

Adding HIL-18 Standard to HCYP2MAG-62K

In the HCYP2MAG-62K kit, the highest HIL-18 Standard concentration will be 5,000 pg/mL. Label a polypropylene microfuge tube 5,000 pg/mL and add 1,240 µL deionized water. Prepare final concentration of HIL-18 Standard by adding 10 µL of the reconstituted 625 ng/mL Human IL-18 Standard to the 5,000 pg/mL. Mix well and use immediately for the following reconstitution.

Reconstitute the lyophilized standard of the HCYP2MAG-62K kit with 250 µL of the 5,000 pg/mL HIL-18 Standard. Invert the vial several times to mix and vortex. Allow the vial to sit for 5-10 minutes. Transfer the reconstituted standard to an appropriately labeled polypropylene microfuge tube. Continue with the standard serial dilution instructions as outlined in the HCYP2MAG-62K kit protocol.

HIL-18 will have the resulting standard curve concentrations in the kits below

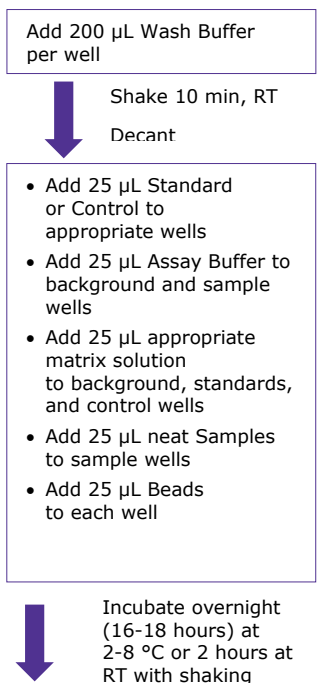
HIL-18 in HCYP2MAG-62K (pg/mL)	HIL-18 in HTH17MAG-14K (pg/mL)
5,000	25,000
1,250	6,250
312.5	1,563
78.1	390.6
19.5	97.7
4.9	24.4
-	6.1

Immunoassay Procedure:
Assaying Single HIL-18 Analyte Only

Note: If combining HIL-18 into another kit, follow that kit's "Immunoassay Procedure". If using the Human IL-18 assay as a singleplex kit, follow the instructions below.

- 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), 1.6 pg/mL, 8 pg/mL, 40 pg/mL, 200 pg/mL, 1,000 pg/mL, 5,000 pg/mL, 25,000 pg/mL], 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.
- If using a filter plate, set the filter plate on a plate holder at all times during reagent dispensing and incubation steps so that the bottom of the plate does not touch any surface.

1. 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).
2. Decant Wash Buffer and remove the residual amount from all wells by inverting the plate and tapping it smartly onto absorbent towels several times.
3. Add 25 µL of each Standard or Control into the appropriate wells. Assay Buffer should be used for 0 pg/mL standard (Background).
4. Add 25 µL of Assay Buffer to the sample wells.
5. Add 25 µL of appropriate matrix solution to the background, standards, and control wells. When assaying serum or plasma, use the Serum Matrix. When assaying tissue culture or other supernatant, use proper control culture medium as the matrix solution.
6. Add 25 µL of Sample (neat) into the appropriate wells.
7. 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.)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).



8. Gently remove well contents and wash plate 3 times following instructions listed in the Plate Washing section.
9. Add 25 µL of Detection Antibodies into each well.
(**Note:** Allow the Detection Antibodies to warm to room temperature prior to addition.)
10. 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.**
11. Add 25 µL Streptavidin-Phycoerythrin to each well containing the 25 µL of Detection Antibodies.
12. Seal, cover with foil and incubate with agitation on a plate shaker for 30 minutes at room temperature (20-25 °C).
13. Gently remove well contents and wash plate 3 times following instructions listed in the Plate Washing section.
14. 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.
15. Run plate on Luminex® 200™, HTS, FLEXMAP 3D®, MAGPIX® with xPONENT® software, or xMAP® INTELLIFLEX with INTELLIFLEX Software.
16. 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:** No sample dilution is required for this assay. If samples were diluted, final sample concentrations should be multiplied by the dilution factor.)

Remove well contents and wash 3X with 200 µL Wash Buffer

Add 25 µL Detection Antibodies per well

Incubate 1 hour at RT

Do Not Aspirate

Add 25 µL Streptavidin-Phycoerythrin per well

Incubate for 30 minutes at RT

Remove well contents and wash 3X with 200 µL Wash Buffer

Add 150 µL Sheath Fluid PLUS or Drive Fluid PLUS per well

Read on Luminex® (100 µL, 50 beads per bead set)

Plate Washing

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

Solid Plate

- 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.

Filter Plate (Cat. No. MX-PLATE)

If using a filter plate, use a vacuum filtration manifold to remove well contents. Wash plate with 200 µL/well of Wash Buffer, removing Wash Buffer by vacuum filtration after each wash. Repeat wash steps as recommended in the Assay Procedure.

Equipment Settings

Luminex® 200™, HTS, FLEXMAP 3D®, MAGPIX® with xPONENT® software and xMAP® INTELLIFLEX 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. LX2RCAL-K25)	Performance Verification Kit (Cat. No. LX2RPVER-K25)
FLEXMAP 3D®	FLEXMAP 3D® Calibrator Kit (Cat. No. F3DCAL-K25)	FLEXMAP 3D® Performance Verification Kit (Cat. No. F3DPVER-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. MPXCAL-K25)	MAGPIX® Performance Verification Kit (Cat. No. MPXPVER-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	HIL-18	62

Quality Controls

The ranges for HIL-18 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.

Note: Quality Control ranges are not provided for HIL-18 when combined in other kits. Ranges apply to singleplex HIL-18 protocol only.

Assay Characteristics

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 = 11 Assays)

Analyte	MinDC (pg/mL)	MinDC+2SD (pg/mL)
HIL-18	1.48	2.17

Precision

Intra-assay precision is generated from the mean of the %CV's from 8 reportable results across two different concentrations of analytes in a single assay. Inter-assay precision is generated from the mean of the %CV's across two different concentrations of analytes across 11 different assays.

Overnight Protocol

Analyte	Intra-assay %CV	Inter-assay %CV
HIL-18	< 5	< 10

Accuracy

Spike Recovery: The data represent mean percent recovery of spiked standards ranging from low, medium, and high concentration in serum matrices (n = 8).

Overnight Protocol

Analyte	% Recovery in Serum Matrix
HIL-18	99

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 Luminex® 200™, adjust probe height to the kit solid plate or to the recommended filter plates using 3 alignment discs. When reading the assay on MAGPIX®, adjust probe height to the kit solid plate or to the recommended filter plates using 2 alignment discs. When reading the assay on FLEXMAP 3D®, adjust probe height to the kit solid plate using 1 alignment disc. For FLEXMAP 3D® 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. When reading the assay on xMAP® INTELLIFLEX, 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 PLUS 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.
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.

Problem	Probable Cause	Solution
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.

FOR FILTER PLATES ONLY

Problem	Probable Cause	Solution
Filter plate will not vacuum	Vacuum pressure is insufficient	Increase vacuum pressure such that 0.2 mL buffer can be suctioned in 3-5 seconds.
	Samples have insoluble particles	Centrifuge samples just prior to assay set-up and use supernatant.
	High lipid concentration	After centrifugation, remove lipid layer and use supernatant.
Plate leaked	Vacuum pressure too high	Adjust vacuum pressure such that 0.2 mL buffer can be suctioned in 3-5 seconds. May need to transfer contents to a new (blocked) plate and continue.
	Plate set directly on table or absorbent towels during incubations or reagent additions	Set plate on plate holder or raised edge so bottom of filter is not touching any surface.
	Insufficient blotting of filter plate bottom causing wicking	Blot the bottom of the filter plate well with absorbent towels after each wash step.
	Pipette touching plate filter during additions	Pipette to the side of plate.
	Probe height not adjusted correctly	Adjust probe to 3 alignment discs in well H6.
	Sample too viscous	May need to dilute sample.

Product Ordering

Replacement Reagents	Cat. No.
Human IL-18 Standard	HIL18-8066
Human IL-18 Quality Control 1 & 2	HIL18-6066
Serum Matrix	HIL18-SM
Bead Diluent	LBD
Human IL-18 Detection Antibody	HIL18-1066
Streptavidin-Phycoerythrin	L-SAPE5
Assay Buffer	L-AB
Set of two 96-Well plates with sealers	MAG-PLATE
10X Wash Buffer	L-WB

Antibody-Immobilized Magnetic Beads

Analyte	Bead No.	Cat. No.
IL-18	62	HIL18-MAG

Well Map

	1	2	3	4	5	6	7	8	9	10	11	12
A	0 pg/mL Standard (Background)	200 pg/mL	QC-1 Control	Etc.								
B	0 pg/mL Standard (Background)	200 pg/mL	QC-1 Control									
C	1.6 pg/mL	1,000 pg/mL	QC-2 Control									
D	1.6 pg/mL	1,000 pg/mL	QC-2 Control									
E	8 pg/mL	5,000 pg/mL	Sample 1									
F	8 pg/mL	5,000 pg/mL	Sample 1									
G	40 pg/mL	25,000 pg/mL	Sample 2									
H	40 pg/mL	25,000 pg/mL	Sample 2									

Notice

We provide information and advice to our customers on application technologies and regulatory matters to the best of our knowledge and ability, but without obligation or liability. Existing laws and regulations are to be observed in all cases by our customers. This also applies in respect to any rights of third parties. Our information and advice do not relieve our customers of their own responsibility for checking the suitability of our products for the envisaged purpose.

The information in this document is subject to change without notice and should not be construed as a commitment by the manufacturing or selling entity, or an affiliate. We assume no responsibility for any errors that may appear in this document.

By purchasing this product, which contains fluorescently labeled microsphere beads authorized by Luminex® Corporation ("Luminex®"), you, the customer, acquire the right under the Luminex® patent rights, if any, to use this product or any portion of this product, including without limitation the microsphere beads contained herein, only with the Luminex® laser based fluorescent analytical test instrumentation marketed under the name of Luminex® 200™, HTS, FLEXMAP 3D®, xMAP® INTELLIFLEX, MAGPIX®.

Contact Information

For the location of the office nearest you, go to SigmaAldrich.com/offices.

Technical Assistance

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

Standard Warranty

The applicable warranty for the products listed in this publication may be found at SigmaAldrich.com/terms.

Safety Data Sheets (SDS)

Safety Data Sheets are available on the product page at SigmaAldrich.com.

Template 00011187FM v. 20.0

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

20335281 Rev 10/25