Human IL-18 Singleplex Magnetic Bead Kit

96-Well Plate Assay

Cat. # HIL18MAG-66K

MILLIPLEX® MAP

HUMAN IL-18 SINGLEPLEX MAGNETIC BEAD KIT
96-Well Plate Assay

 # HIL18MAG-66K

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For Research Use Only. Not for Use in Diagnostic Procedures.

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.

MILLIPLEX® MAP 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 validation process include: dilution linearity, kit stability, and sample behavior (e.g. detectability and stability).

Each MILLIPLEX® MAP 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® MAP 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.

EMD Millipore's MILLIPLEX® MAP 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 validate and build the most comprehensive library available for protein detection and quantitation.
EMD Millipore's MILLIPLEX® MAP 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® MAP Human IL-18 Singleplex Magnetic Bead Kit can be combined and assayed within the following kits:

- MILLIPLEX® MAP Human Cytokine/Chemokine Magnetic Bead Panel Catalog# HCYTOMAG-60K (or HCYTMAG-60K-PX29, HCYTMAG-60K-PX30, HCYTMAG-60K-PX38, HCYTMAG-60K-PX41, or the corresponding bulk format kits)
- MILLIPLEX® MAP Human Cytokine/Chemokine Magnetic Bead Panel II Catalog# HCYP2MAG-62K (with the exception of the CTACK/CCL27 assay, see below for further information)
- MILLIPLEX® MAP Human Th17 Magnetic Bead Panel Catalog# HTH17MAG-14K (or HT17MG-14K-PX25, or the corresponding bulk format kit)

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Please read entire protocol before use.

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

PRINCIPLE

MILLIPLEX® MAP is 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® uses 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.
- EMD Millipore provides three Luminex® instruments to acquire and analyze data using two detection methods:
    - The Luminex® analyzers Luminex® 200™ and FLEXMAP 3D®, 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. EMD Millipore combines 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.

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

Note: Store all reagents at 2 – 8°C

Reagents Supplied	Catalog Number	Volume	Quantity
**Human IL-18 Standard (25X)	**HIL18-8066	Lyophilized	1 vial
Human IL-18 Quality Controls 1 and 2 (25X)	HIL18-6066	Lyophilized	1 vial each
***Serum Matrix** Note: Contains 0.08% Sodium Azide	***HIL18-SM**	Lyophilized	1 bottle
Bead Diluent	LBD	3.5 mL	1 bottle
Set of one 96-Well Plate with 2 sealers	----------	----------	1 plate 2 sealers
Assay Buffer	L-AB	30 mL	1 bottle
10X Wash Buffer Note: Contains 0.05% Proclin	L-WB	60 mL	1 bottle
Human IL-18 Detection Antibodies (20X)	HIL18-1066	200 µL	1 vial
***Streptavidin-Phycoerythrin**	***L-SAPE5**	5.5 mL	1 bottle
Mixing Bottle	----------	----------	1 bottle

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

**The MILLIPLEX® MAP 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. #	Volume	Concentration in vial
Anti-Human IL-18 Bead	62	HIL18-MAG	90 µL	50X

MATERIALS REQUIRED BUT NOT PROVIDED

Reagents

1. Luminex® Sheath Fluid (EMD Millipore Catalog # SHEATHFLUID) or Luminex® Drive Fluid (EMD Millipore Catalog # MPXDF-4PK)

Instrumentation / Materials

1. Adjustable Pipettes with Tips capable of delivering 25 µL to 1000 µL
2. Multichannel Pipettes capable of delivering 5 µL to 50 µL or 25 µL to 200 µL
3. Reagent Reservoirs
4. Polypropylene Microfuge Tubes
5. Polypropylene Conical Tube capable of accommodating > 3.2 mL
6. Rubber Bands
7. Aluminum Foil
8. Absorbent Pads
9. Laboratory Vortex Mixer
10. Sonicator (Branson Ultrasonic Cleaner Model # B200 or equivalent)
11. Titer Plate Shaker (VWR® Microplate Shaker Cat # 12620-926 or equivalent)
12. Luminex® 200™, HTS, FLEXMAP 3D®, or MAGPIX® with xPONENT® software by Luminex® Corporation
13. Automatic Plate Washer for magnetic beads (BioTek® 405 LS and 405 TS, EMD Millipore Catalog #40-094, # 40-095, # 40-096, # 40-097 or equivalent) or Handheld Magnetic Separation Block (EMD Millipore Catalog # 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 (EMD Millipore Catalog # MX-PLATE) to run the assay using a Vacuum Filtration Unit (EMD Millipore Vacuum Manifold Catalog # MSVMHTS00 or equivalent with EMD Millipore Vacuum Pump Catalog # 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.

Note: See Full Labels of Hazardous components on next page.

Full labels of hazardous components in this kit:

Ingredient, Cat #		Full 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-SAPE6		**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 Luminex® 200™, adjust probe height according to the protocols recommended by Luminex® to the kit solid plate or to the recommended EMD Millipore filter plates using 3 alignment discs. When reading the assay on MAGPIX®, adjust probe height according to the protocols recommended by Luminex® to the kit solid plate or to the recommended EMD Millipore filter plates using 2 alignment discs. When reading the assay on FLEXMAP 3D®, adjust probe height according to the protocols recommended by Luminex® 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 in each well and 75 µL should be aspirated.
- 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.

TECHNICAL GUIDELINES (continued)

- 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.

A. Preparation of Serum Samples:
   - Allow the blood to clot for at least 30 minutes before centrifugation for 10 minutes at 1000xg. 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.

B. Preparation of Plasma Samples:
   - Plasma collection using EDTA as an anti-coagulant is recommended. Centrifuge for 10 minutes at 1000xg 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.

C. 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 "*PREPARATION 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.*

A. 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.)

B. 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.

C. 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. 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.

D. 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.

E. 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.

F. 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. 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.

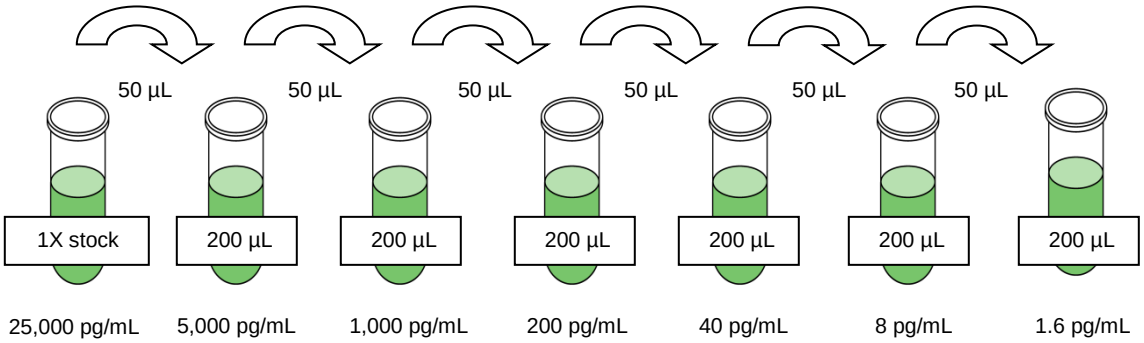
2.) Preparation of Working Standards

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	Volume of Deionized Water to Add	Volume of Standard to Add
625,000 pg/mL	200 µL	0

Standard Concentration (pg/mL)	Volume of Assay Buffer to Add	Volume of Standard to Add
25,000	240 µL	10 µL of 625,000 pg/mL
5,000	200 µL	50 µL of 25,000 pg/mL
1,000	200 µL	50 µL of 5,000 pg/mL
200	200 µL	50 µL of 1,000 pg/mL
40	200 µL	50 µL of 200 pg/mL
8	200 µL	50 µL of 40 pg/mL
1.6	200 µL	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 (I.E. "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® MAP Human Cytokine/Chemokine Magnetic Bead Panel Catalog# HCYTOMAG-60K (or HCYTMAG-60K-PX29, HCYTMAG-60K-PX30, HCYTMAG-60K-PX38, HCYTMAG-60K-PX41, or the corresponding bulk format kits)
- MILLIPLEX® MAP Human Cytokine/Chemokine Magnetic Bead Panel II Catalog# HCYP2MAG-62K (with the exception of the CTACK/CCL27 assay, see below for further information)
- MILLIPLEX® MAP Human Th17 Magnetic Bead Panel Catalog# HTH17MAG-14K (or HT17MG-14K-PX25, or the corresponding bulk format kit)

A. 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.

B. 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.

C. 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.

Adding HIL-18 Quality Controls to HCYTOMAG-60K or 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.

D. 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.

E. 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.

F. 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.

Adding HIL-18 Standard to HCYTOMAG-60K or 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. Continue with the standard serial dilution instructions as outlined in the HCYTOMAG-60K or 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. 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:

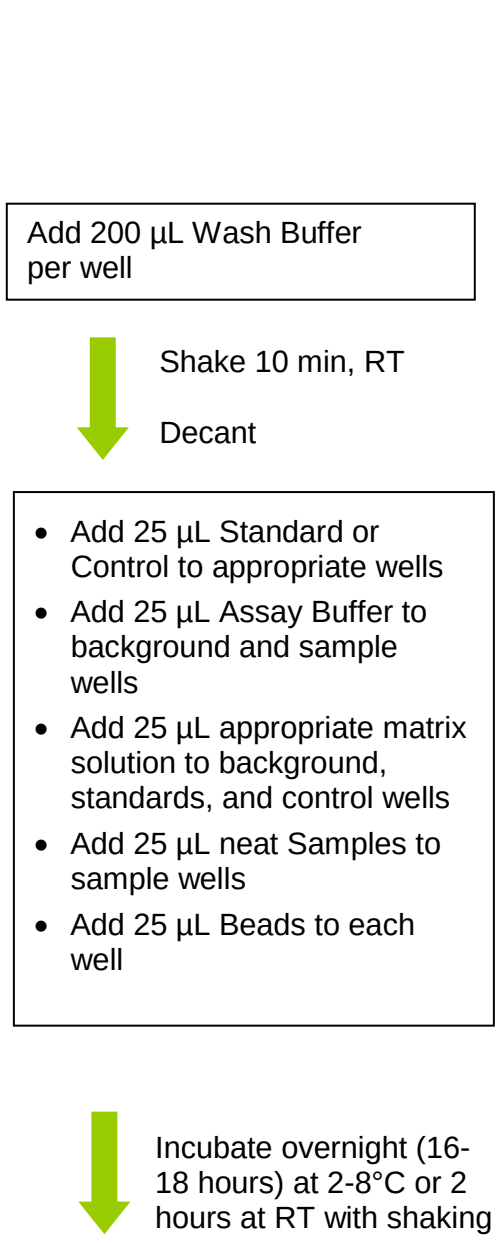
HIL-18 in HCYTOMAG-60K (pg/mL)	**HIL-18 in HCYP2MAG-62K (pg/mL)**	**HIL-18 in HTH17MAG-14K (pg/mL)**
25,000	5,000	25,000
5,000	1,250	6,250
1,000	312.5	1,563
200	78.1	390.6
40	19.5	97.7
8	4.9	24.4
--	--	6.1

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.)
8. 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).
9. Gently remove well contents and wash plate 3 times following instructions listed in the **PLATE WASHING** section.



10. Add 25 µL of Detection Antibodies into each well. (Note: Allow the Detection Antibodies to warm to room temperature prior to addition.)
11. 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.**
12. Add 25 µL Streptavidin-Phycoerythrin to each well containing the 25 µL of Detection Antibodies.
13. Seal, cover with foil and incubate with agitation on a plate shaker for 30 minutes at room temperature (20-25°C).
14. Gently remove well contents and wash plate 3 times following instructions listed in the **PLATE WASHING** section.
15. Add 150 µL of Sheath Fluid (or Drive Fluid if using MAGPIX®) to all wells. Resuspend the beads on a plate shaker for 5 minutes.
16. Run plate on Luminex® 200™, HTS, FLEXMAP 3D® or MAGPIX® with xPONENT® software.
17. 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.)

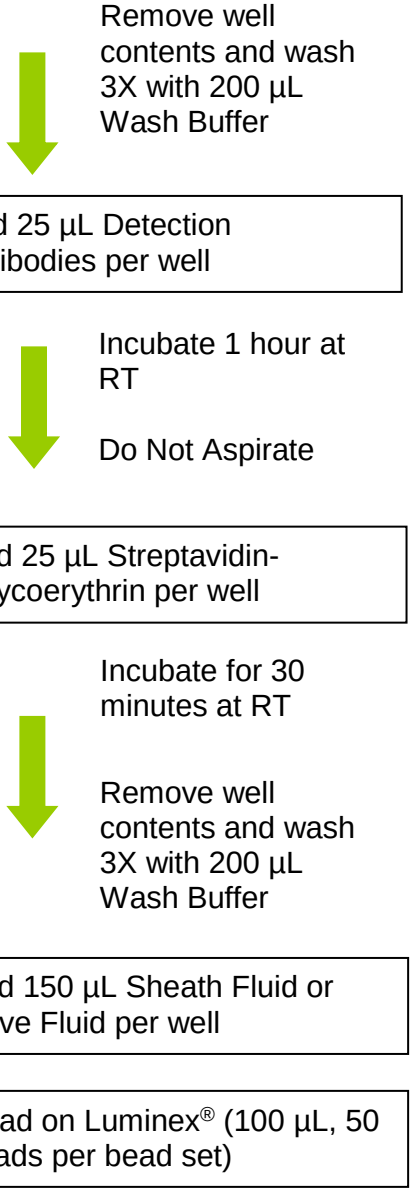


PLATE WASHING

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

1.) Solid Plate

A.) Handheld magnet **(EMD Millipore Catalog #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.

B.) Magnetic plate washer **(EMD Millipore Catalog #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.

2.) Filter Plate (EMD Millipore Catalog #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®, and MAGPIX® with xPONENT® software:

These specifications are for the Luminex® 200™, Luminex® HTS, Luminex® FLEXMAP 3D®, and Luminex® MAGPIX® with xPONENT® software. Luminex® instruments with other software (e.g. 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, the Luminex® 200™ and HTS instruments must be calibrated with the xPONENT® 3.1 compatible Calibration Kit (EMD Millipore Catalog # 40-275) and performance verified with the Performance Verification Kit (EMD Millipore Catalog # 40-276). The Luminex® FLEXMAP 3D® instrument must be calibrated with the FLEXMAP 3D® Calibrator Kit (EMD Millipore Catalog # 40-028) and performance verified with the FLEXMAP 3D® Performance Verification Kit (EMD Millipore Catalog # 40-029). The Luminex® MAGPIX® instrument must be calibrated with the MAGPIX® Calibration Kit (EMD Millipore Catalog # 40-049) and performance verified with the MAGPIX® Performance Verification Kit (EMD Millipore Catalog # 40-050).

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.

EQUIPMENT SETTINGS (continued)

The Luminex® probe height must be adjusted to the plate provided in the kit. Please use Catalog #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 the EMD Millipore website emdmillipore.com using the catalog 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.

Analyte	Overnight Protocol (n = 11 Assays)	
	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.

Analyte	Overnight Protocol	
	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).

Analyte	Overnight Protocol
	% Recovery in Serum Matrix
HIL-18	99

TROUBLESHOOTING GUIDE

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 EMD Millipore filter plates using 3 alignment discs. When reading the assay on MAGPIX®, adjust probe height to the kit solid plate or to the recommended EMD Millipore 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 in each well and 75 µL should be aspirated.
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 (e.g. interleukin modified tissue culture medium).
	Insufficient washes	Increase number of washes.
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 (e.g. 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.

Problem	Probable Cause	Solution
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 (e.g. 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.

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.

REPLACEMENT REAGENTS	**Catalog #**
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 #*	*Cat. #*
IL-18	62	HIL18-MAG

ORDERING INFORMATION

To place an order or to obtain additional information about our immunoassay products, please contact your Customer Service or Technical Support Specialist.

Contact information for each region can be found on our website:

emdmillipore.com/contact

Conditions of Sale

For Research Use Only. Not for Use in Diagnostic Procedures.

Safety Data Sheets (SDS)

Safety Data Sheets for EMD Millipore products may be downloaded through our website at emdmillipore.com/msds.

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									