

Human Cytokine Magnetic 25-Plex Panel

Catalog Number LHC0009M (100 tests)

Pub. No. MAN0003646 Rev. 4.0 (00)

CAUTION! This kit contains materials with small quantities of sodium azide. Sodium azide reacts with lead and copper plumbing to form explosive metal azides. Upon disposal, flush drains with a large volume of water to prevent azide accumulation. Avoid ingestion and contact with eyes, skin and mucous membranes. In case of contact, rinse affected area with plenty of water. Observe all federal, state, and local regulations for disposal.

WARNING! Read the Safety Data Sheets (SDSs) and follow the handling instructions. Wear appropriate protective eyewear, clothing, and gloves. Safety Data Sheets (SDSs) are available from thermofisher.com/support.

Product description

The Human Cytokine Magnetic 25-Plex Panel contains all necessary reagents for use with Luminex™ 100™, Luminex™ 200™, FLEXMAP 3D™, or MAGPIX™ instrumentation. The xPONENT™ software package is recommended for data analysis.

Note: Only certain upgraded software versions on the Luminex™ 100™ instrument are compatible with the magnetic kit.

The Human Cytokine Magnetic 25-Plex Panel is designed for the quantitative determination of IL-1β, IL-1RA, IL-2, IL-2R, IL-4, IL-5, IL-6, IL-7, IL-8, IL-10, IL-12 (p40/p70), IL-13, IL-15, IL-17, TNF-α, IFN-α, IFN-γ, GM-CSF, MIP-1α, MIP-1β, IP-10, MIG, Eotaxin, RANTES, and MCP-1 in human serum, plasma, and tissue culture supernatant.

Note: The three sample types have been validated, but other sample types may be used.

To find out more details regarding use of the kit, contact Technical Support at techsupport@lifetech.com.

Note: All reagents in this kit have been optimized for use with flat bottom plates. For any questions, please contact Technical Support.

Contents and storage

Upon receipt, store the kit at 2°C to 8°C. **Do not freeze.**

Contents	Amount
Human Cytokine Magnetic 25-Plex Antibody Beads (1X); contains 0.05% sodium azide, light-sensitive	2.5 mL
Human Cytokine Magnetic 25-Plex Biotinylated Antibody Concentrate (10X); contains 0.1% sodium azide	1 mL
Human Cytokine 16-Plex Standard; contains 0.1% sodium azide	2 vials
Human Cytokine 14-Plex Standard; contains 0.1% sodium azide	2 vials
Wash Solution Concentrate (20X); contains 0.1% sodium azide	50 mL
Incubation Buffer; contains 0.05% sodium azide	12 mL
Assay Diluent; contains 0.1% sodium azide	15 mL
Biotin Diluent; contains 3.3 mM Thymol	12 mL
Streptavidin-RPE Concentrate (10X); contains 0.1% sodium azide, light-sensitive	1 mL
Streptavidin-RPE Diluent; contains 3.3 mM Thymol	12 mL
96-well Flat Bottom Plate	1 × 96-well plate
Plate Cover	1 cover

Materials required but not supplied

- Luminex™ 100™, Luminex™ 200™, FLEXMAP 3D™, or MAGPIX™ system with data acquisition and analysis software
- Calibrated adjustable precision pipettes and polypropylene tubes for diluting solutions
- Vortex mixer and orbital shaker
- Magnetic 96-Well Separator (Cat. No. A14179, EPX-55555-000, or equivalent), or automated plate washer
- Sonicating water bath

Procedural guidelines

- Do not invert the plate during the assay unless the plate is on the magnetic separator.
- The fluorescent beads and RPE reagents are light sensitive, and should be protected from light.
- Handle all blood components and biological materials as potentially hazardous.
- Reagents are lot-specific. Do not mix or interchange different reagent lots from various kit lots.
- Set the orbital shaker to a speed providing optimum agitation without the liquid splashing onto the lid. For an orbital shaker with a 3-mm orbital radius, a speed between 500 and 600 rpm is recommended.
- The magnetic beads settle rapidly. It is therefore important to mix well prior to use.

Before you begin

- Allow all reagents to warm to room temperature before use. Mix all reagents before use.
- Prepare a plate plan for your assay. Standards, samples, and in-house controls should be run in duplicate. It is recommended to include in-house controls with every assay.

Prepare 1X Wash Solution

Add 50 mL of Wash Solution Concentrate (20X) to 950 mL of deionized water. Mix well.

Note: Precipitate in the Wash Solution Concentrate (20X) can be dissolved by warming the bottle in a 37°C water bath and mixing until the precipitate is dissolved.

Note: 1X Wash Solution is stable for up to 2 weeks when stored at room temperature.

Sample preparation guidelines

- Collect samples in pyrogen/endotoxin-free tubes. Centrifuge, separate, and transfer samples to polypropylene tubes for storage.
- Avoid the use of hemolyzed or lipemic sera.
- Freeze samples after collection if samples will not be tested immediately. Avoid multiple freeze-thaw cycles of frozen samples. Thaw completely and mix well (do not vortex) prior to analysis.
- Clarify samples by centrifugation (12,000–16,000 × g for 10 minutes at 2°C to 8°C) prior to analysis.

Dilute samples

Samples can be used directly for the assay.

- If sample concentrations exceed the standard curve, dilute samples further and reanalyze:
 - **Serum or plasma** – Dilute samples in Assay Diluent.
 - **Tissue culture supernatant** – Dilute in the corresponding tissue culture medium.

Standard reconstitution guidelines

Each kit comes with two identical vials of standard, so that two separate runs can be made with freshly prepared standards.

- Reconstitute protein standard(s) within 1 hour of use. **Once reconstituted, standards cannot be stored for future use.**
- Reconstitute the standard with the appropriate diluent:
 - **Serum or plasma** samples—reconstitute with the provided Assay Diluent.
 - **Other sample types** (e.g., tissue culture supernatant)—reconstitute with a mixture of 50% Assay Diluent and 50% of a matrix which closely resembles that of the sample type (50/50 diluent mixture).
- **Do not vortex.** Avoid formation of foam when mixing or reconstituting protein solutions.

Reconstitute lyophilized standard

For this 25-plex assay, mix and reconstitute one vial each of Human 16-Plex Standard and Human 14-Plex Standard as follows:

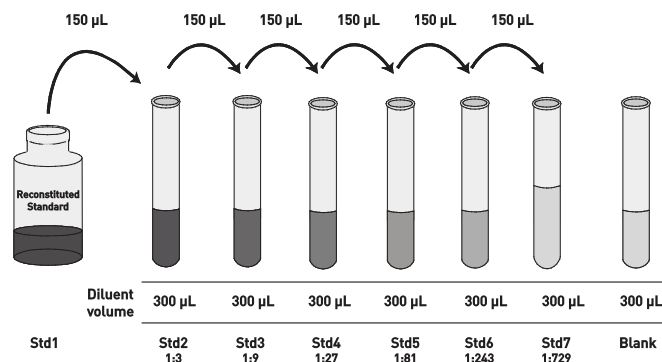
1. Add the volume of appropriate diluent as indicated on the vial label/certificate of analysis and incubate for 10 minutes.
2. Gently mix the standard to ensure complete reconstitution, and incubate at room temperature for an additional 5 minutes.
3. After the two vials are reconstituted, combine the contents of the two vials.

Note: Some reduction of volume may occur during transfer of material, but the amount should be sufficient to perform your experiment.

Prepare standard curve

Run a standard curve with each assay. Perform a serial dilution of the reconstituted standard(s) in **polypropylene tubes**.

1. Add 300 µL Assay Diluent (for serum and plasma samples), or 300 µL of a 50/50 diluent mixture (for other sample types) to each of 7 tubes.
2. Add 150 µL from one tube to the next one to make 1:3 serial dilutions of the standard. Mix thoroughly and change pipette tips between steps.



3. Discard any remaining reconstituted standard.

Wash procedure

- Separate the plate from the magnetic separator before adding wash solution or any reagent to the plate.
 - Place the plate on the magnetic separator before decanting liquid.
 - Incomplete washing adversely affects assay results. Perform all wash steps with the 1X Wash Solution.
1. Add 1X Wash Solution to the beads in the assay wells.
 2. Place the 96-well flat bottom plate containing the beads onto the magnetic separator.
 3. Allow the beads to settle for 30–60 seconds.
 4. Turn the magnetic separator and plate (held securely together) upside down, decant the fluid, and blot excess liquid on a stack of dry paper towels.

Note: Blotting excess liquid is important to avoid cross contamination from droplets.

Assay procedures

Total assay time is 3.5 hours.

IMPORTANT! Perform a standard curve with each assay.

IMPORTANT! Always keep beads and plate protected from light.

Note: All volumes used for preparing 1X solutions are calculated with a 1:11 dilution factor to provide extra pipetting volume.

Capture analytes

1. Determine the number of wells in the 96-well plate to be used in the assay.
2. Vortex the 1X Antibody Beads for 30 seconds, then sonicate for 30 seconds immediately before use.
3. Add 25 μ L of 1X Antibody Beads into each assay well. Protect the plate from light once the beads have been added.
4. Wash assay wells twice with 200 μ L of 1X Wash Solution (see "Wash procedure").
5. Add 50 μ L of Incubation Buffer into each assay well.
6. Add 100 μ L of diluted standards into standard wells.
7. Add 100 μ L of your blank into blank/background wells.
8. Add 50 μ L of Assay Diluent followed by 50 μ L of sample in sample wells.
9. Cover and incubate the plate for 2 hours at room temperature under agitation on an orbital plate shaker.

Note: (Optional) the plate can be incubated under agitation on an orbital shaker overnight at 2°C to 8°C.

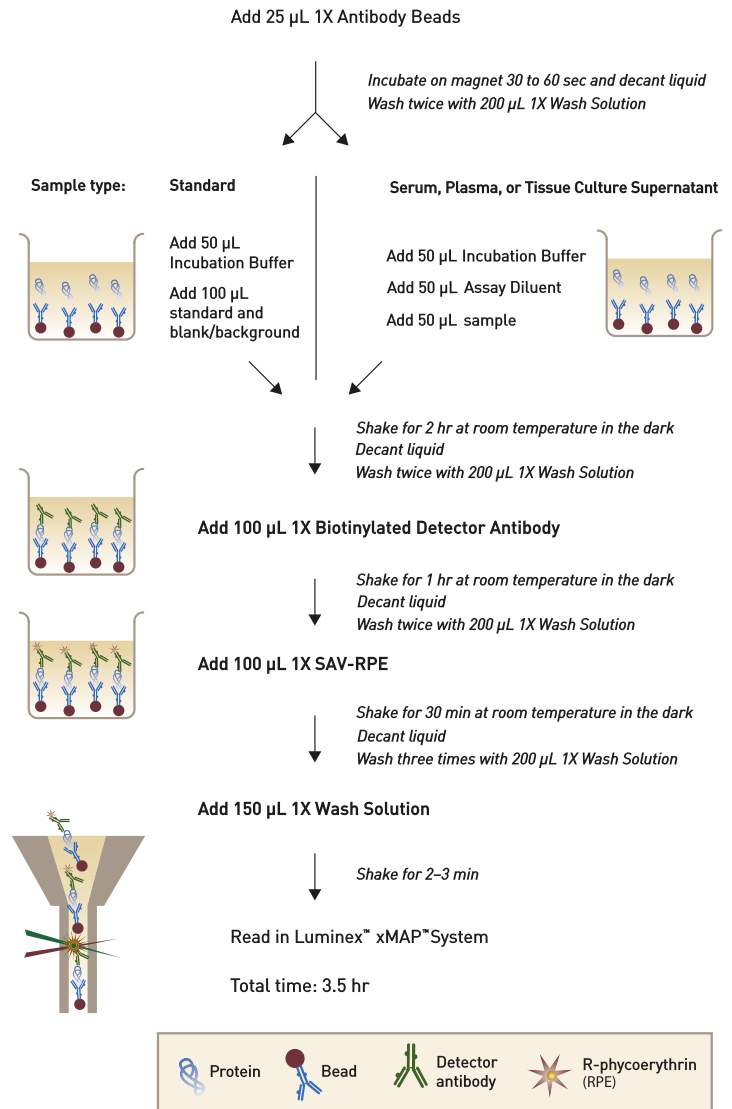
Detect analytes

1. Prepare 1X Biotinylated Detector Antibody in a conical tube. Scale volumes according to the number of assay wells needed. For a single assay well, add:
 - 100 μ L of Biotin Diluent
 - 10 μ L of 10X Biotinylated Antibody
2. Decant liquid and wash the wells twice with 200 μ L 1X Wash Solution (see "Wash procedure").
3. Add 100 μ L 1X Biotinylated Detector Antibody to all assay wells. Cover and incubate the plate for 1 hour at room temperature on an orbital plate shaker.
4. Prepare 1X Streptavidin-RPE solution in a conical tube. Scale volumes according to the number of assay wells needed. For a single assay well, add:
 - 100 μ L of RPE-Diluent
 - 10 μ L of 10X Streptavidin-RPE (protect from light)
5. Decant liquid and wash the wells twice with 200 μ L 1X Wash Solution (see "Wash procedure").
6. Add 100 μ L 1X Streptavidin-RPE solution to each assay well. Cover and incubate the plate for 30 minutes at room temperature on an orbital plate shaker.
7. Decant liquid and wash the wells 3 times with 200 μ L 1X Wash Solution (see "Wash procedure").

Read assay results

1. Add 150 μ L 1X Wash Solution to each assay well and place the plate on an orbital plate shaker for 2–3 minutes prior to analysis.
Note: If the plate cannot be read on the day of the assay, cover and store the plate in the dark at 2°C to 8°C for reading the following day without significant loss of fluorescent intensity. The next day prior to reading, remove 1X Wash Solution from stored plates, and begin "Read assay results" procedure starting from step 1.
2. Uncover the plate and insert the plate into the XY platform of the Luminex™ 100™, Luminex™ 200™, FLEXMAP 3D™, or MAGPIX™ instrument, and analyze the samples. Determine the concentration of samples from the standard curve using curve fitting software.

Workflow



Instrument setup

Note: Before running the assay, set the probe height appropriately, and calibrate and verify the system.

Instrument	MAGPIX™	FLEXMAP 3D™	Luminex™ 100™/200™
Probe height	Set to appropriate plate	Set to appropriate plate	N/A
Bead type	MagPlex™	MagPlex™	MagPlex™
Volume	75 µL ^[1]	75 µL	75 µL
Timeout	N/A	50 seconds	50 seconds
Doublet discriminator	N/A	7,800–20,000	7,800–20,000
Plate heater	Off	Off	Off
PMT	N/A	Default (High)	Default (Low)
Standard curve	Quantitative	Quantitative	Quantitative
Target bead count	100	100	100
Algorithm	Default (5 PL Logistic Weighted)	Default (5 PL Logistic Weighted)	Default (5 PL Logistic Weighted)
Sample dilution	1:2	1:2	1:2
Standard dilution	1:3	1:3	1:3
Standard concentration	See the lot specific technical data sheet included with kit		

^[1] Volume can be adjusted during acquisition to optimize bead count.

xPONENT™ software result guidelines

- Confirm target bead counts reached for all analytes in each well to assure accurate data acquisition.
- View the standard curve and individual analyte statistics.
- Individual analyte PDF report with standard curves can be generated using the **Reports** tab found under **Results**.
- Qualify all the standard points by checking for inaccurate standard points due to excessive plateauing or bottoming out.
 - Net MFI (MFI – background) and %Recovery limits are useful checks for the bottom and top of the curve.
 - Use **Invalidate**, **Analyze** and **Save** to remove inaccurate data points (plateaus or bottom outs). Review %CV of replicates.
 - Note that %CV of replicates calculation is based on concentration, not MFI data.
- Close results and use **Export CSV** to export data to Excel™ readable file.

Limited product warranty

Life Technologies Corporation and/or its affiliate(s) warrant their products as set forth in the Life Technologies' General Terms and Conditions of Sale found on Life Technologies' website at www.thermofisher.com/us/en/home/global/terms-and-conditions.html. If you have any questions, please contact Life Technologies at www.thermofisher.com/support.

Product label explanation of symbols and warnings



Manufacturer's address: Bender MedSystems GmbH | Campus Vienna Biocenter 2 | 1030 Vienna, Austria

The information in this guide is subject to change without notice.

DISCLAIMER: TO THE EXTENT ALLOWED BY LAW, LIFE TECHNOLOGIES AND/OR ITS AFFILIATE(S) WILL NOT BE LIABLE FOR SPECIAL, INCIDENTAL, INDIRECT, PUNITIVE, MULTIPLE, OR CONSEQUENTIAL DAMAGES IN CONNECTION WITH OR ARISING FROM THIS DOCUMENT, INCLUDING YOUR USE OF IT.

Important Licensing Information: These products may be covered by one or more Limited Use Label Licenses. By use of these products, you accept the terms and conditions of all applicable Limited Use Label Licenses.

Corporate entity: Life Technologies Corporation | Carlsbad, CA 92008 USA | Toll Free in USA 1 800 955 6288

Trademarks: All trademarks are the property of Thermo Fisher Scientific and its subsidiaries unless otherwise specified. FLEXMAP 3D, MicroPlex, xPONENT, Luminex, Luminex 100, and Luminex 200 are trademarks of Luminex Corporation. Excel is a trademark of Microsoft Corporation.

©2017 Thermo Fisher Scientific Inc. All rights reserved. All trademarks are the property of Thermo Fisher Scientific and its subsidiaries unless otherwise specified. MAGPIX, FLEXMAP 3D, MagPlex, xPONENT, Luminex, Luminex 100, and Luminex200 are trademarks of Luminex Corporation. Excel is a trademark of Microsoft Corporation.