Human Ultrasensitive Cytokine Magnetic 10-Plex Panel

Catalog Number LHC6004M (100 tests)

Pub. No. MAN0009868 **Rev.** 3.0 (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 Ultrasensitive Cytokine Magnetic 10-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 Ultrasensitive Cytokine Magnetic 10-Plex Panel is designed for the quantitative determination of GM-CSF, IFN-γ, IL-1β, IL-2, IL-4, IL-5, IL-6, IL-8, IL-10, and TNF-α in 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 Ultrasensitive Cytokine Magnetic 10-Plex Antibody Bead Concentrate (10X); contains 0.05% sodium azide, light-sensitive	0.25 mL
Human Ultrasensitive Cytokine Magnetic 10-Plex Biotinylated Antibody Concentrate (10X); contains 0.1% sodium azide	1 mL
Human Ultrasensitive Cytokine 10-plex Standard; contains 0.1% sodium azide	2 vials
Wash Solution Concentrate (20X); contains 0.1% sodium azide	50 mL
Ultrasensitive Assay Diluent; contains 0.1% sodium azide	15 mL
Ultrasensitive Standard Reconstitution Buffer	3 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
Black Lid Cover	1 lid

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 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 Buffer

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

- If sample concentrations exceed the standard curve, dilute samples further and reanalyze:
  - **Serum and 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 each 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 standard

For this 10-plex assay, mix and reconstitute one of the standard vials in Ultrasensitive Standard Reconstitution Buffer as follows:

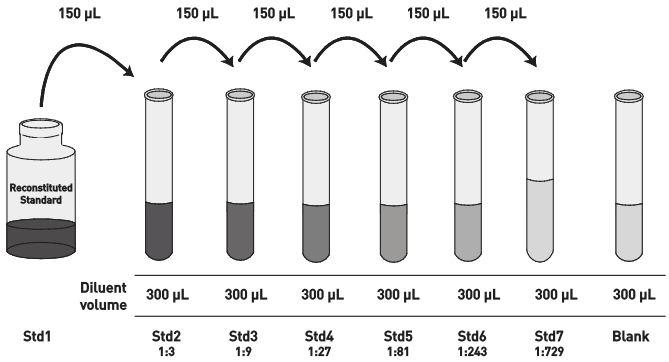
1. Add the volume of Ultrasensitive Standard Reconstitution Buffer indicated on the vial label and incubate for 10 minutes.
2. Gently mix the standard to ensure complete reconstitution, and incubate at room temperature for an additional 5 minutes.
3. If working with serum or plasma samples, dilute the reconstituted standard 1:30 in Ultrasensitive Assay Diluent. When working with other sample types, such as tissue culture supernatants, perform this dilution in a solution composed of 50% Ultrasensitive Assay Diluent and 50% matrix that matches the sample matrix.
4. Perform a 1:3 dilution of the reconstituted and diluted standard before preparing the serial dilutions for the standard curve.

Prepare standard curve

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

Note: Further dilutions to the standard curve may be added to assist in low end detection.

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 Antibody Bead Concentrate (10X) for 30 seconds, then sonicate for 30 seconds immediately before use.
3. Prepare 1X Antibody Beads in a conical tube. Scale volumes according to the number of assay wells needed. For a single assay well, add:
   - 25 µL of 1X Wash Solution
   - 2.5 µL of vortexed Antibody Bead Concentrate (10X)
4. Vortex the 1X Antibody Beads for 30 seconds, then sonicate for 30 seconds immediately before use.
5. Add 25 µL of 1X Antibody Beads into each assay well. Protect the plate from light once the beads have been added.
6. Wash assay wells twice with 200 µL of 1X Wash Solution (see "Wash procedure").
7. Add 100 µL of diluted standards into standard wells.
8. Add 100 µL of your blank into blank/background wells.
9. Add 50 µL of Assay Diluent followed by 50 µL of sample in sample wells.
10. Cover with an opaque lid 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

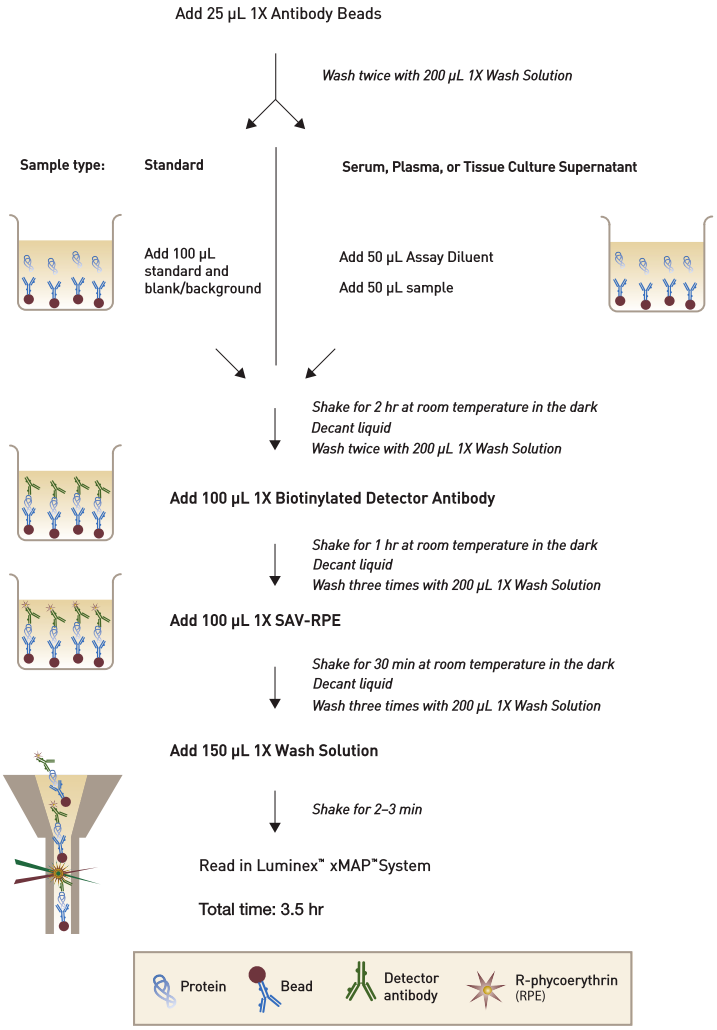
1. Prepare 1X Biotinylated Detector Antibody in a conical tube. Scale volumes according to the number of assay wells needed. For a single assay well, add:
   - 100 µL of Biotin Diluent
   - 10 µL of 10X Biotinylated Antibody
2. Decant liquid and wash the wells twice with 200 µL 1X Wash Solution (see "Wash procedure").
3. Add 100 µL 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 µL of RPE-Diluent
   - 10 µL of 10X Streptavidin-RPE (protect from light)
5. Decant liquid and wash the wells twice with 200 µL 1X Wash Solution (see "Wash procedure").
6. Add 100 µL 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 µL 1X Wash Solution (see "Wash procedure").

Read assay results

1. Add 150 µL 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™	Luminex™ 100™/200™	FLEXMAP 3D™
Probe height	Set to appropriate plate	N/A	Set to appropriate plate
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 (Low)	Default (High)
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 or as appropriate	1:2 or as appropriate	1:2 or as appropriate
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.