Human Cytokine Magnetic 35-Plex Panel

Catalog no. LHC6005M **Quantity:** 100 tests

Pub. No. MAN0015681 **Rev 2.0 (00)**

Description

The Human Cytokine Magnetic 35-Plex Panel contains all necessary reagents for use with Luminex™ 100™, 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. To find out more, contact Technical Support at techsupport@lifetech.com.

The Human Cytokine Magnetic 35-Plex Panel is designed for the quantitative determination of EGF, Eotaxin, FGF basic, G-CSF, GM-CSF, HGF, IFN-α, IFN-γ, IL-1ra, IL-1α, IL-1β, IL-2, IL-2r, IL-3, IL-4, IL-5, IL-6, IL-7, IL-8, IL-9, IL-10, IL-12 (p40/p70), IL-13, IL-15, IL-17A, IL-17F, IL-22, IP-10, MCP-1, MIG, MIP-1α, MIP-1β, RANTES, TNF-α, and VEGF in serum, plasma, and tissue culture supernatant (the three sample types have been validated but other sample types may be used).

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

The components included in the kit are listed below. Upon receipt, store the kit at 2°C to 8°C. **Do not freeze.**

Components	Quantity
Human Cytokine Magnetic 35-Plex Antibody Bead Solution (1X) (contains 0.05% sodium azide), light-sensitive	2.5 mL
Human Cytokine Magnetic 35-Plex Biotinylated Antibody Concentrate (10X) (contains 0.1% sodium azide)	0.5 mL
Human 20-plex Standard (contains 0.1% sodium azide)	2 vials
Human 25-plex Standard (contains 0.1% sodium azide)	2 vials
Human 4-plex Standard (contains 0.1% sodium azide)	2 vials
Wash Solution Concentrate, (20X) (contains 0.1% sodium azide)	50 mL
Assay Diluent (contains 0.1% sodium azide)	15 mL
Incubation Buffer (contains 0.05% sodium azide)	12 mL
Biotin Diluent (contains 3.3 mM Thymol)	12 mL
Streptavidin RPE Concentrate (10X) (contains 0.1% sodium azide), light-sensitive	0.5 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

CAUTION! This kit contains materials with small quantities of sodium azide. Sodium azide may react with lead and copper plumbing to form highly explosive metal azides.

Materials required but not provided

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

General 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 extensive exposure to 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.

For Research Use Only. Not for use in diagnostic procedures.

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*	75 µL	75 µL
Timeout	N/A	50 sec	50 sec
Doublet Discriminator	N/A	7800–20,000	7800–20,000
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	Refer to the lot specific technical data sheet included with kit		

*Volume can be adjusted during acquisition to optimize bead count

xPONENT® software results

- 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

REF	Catalog Number	LOT	Batch code		Temperature limitation		Use by		Manufacturer		Consult instructions for use		Caution, consult accompanying documents

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

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

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Before starting

- Allow all reagents to warm to room temperature 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 wash solution

Prepare 1X Wash Solution by adding 50 mL of Wash Solution Concentrate (20X) to 950 mL of deionized water. Mix well.

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

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.

General 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.

Prepare sample

Samples can be used directly for the assay. However, if the sample concentrations exceed the standard curve, dilute samples further and reanalyze.

- Dilute serum and plasma samples in Assay Diluent.
- Dilute tissue culture supernatants in the corresponding tissue culture medium.

Reconstitute lyophilized standards

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

- Reconstitute protein standard(s) within 1 hour of use.

  Note: Reconstituted standards cannot be stored for future use.
- When using serum or plasma samples, reconstitute the standard with Assay Diluent provided.
- If using other sample types (e.g., tissue culture supernatant), reconstitute the standard with a mixture, composed of 50% Assay Diluent and 50% of the matrix which closely resembles the sample type (50/50 diluent mixture).
- **Do not vortex**. Avoid formation of foam when mixing or reconstituting protein solutions.

Reconstitute lyophilized standards, continued

For this 35-plex assay, mix and reconstitute one vial each of Human 20-Plex Standard, Human 25-Plex Standard, and Human 4-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 all 3 vials are reconstituted, combine the contents of the 3 vials (total volume of 1 mL).

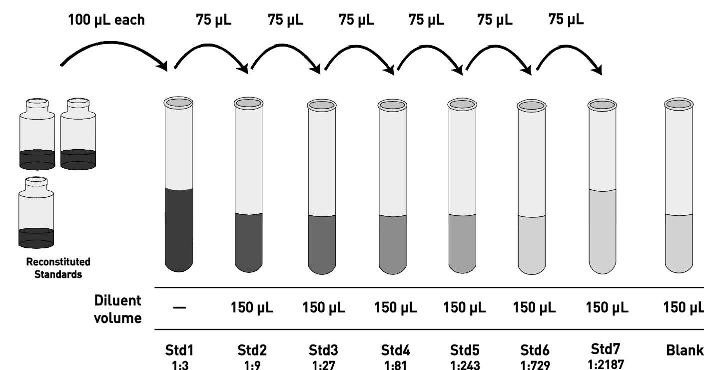
   Note: Some reduction of volume may occur during transfer of material, but the amount should be sufficient to perform your experiment.

Prepare standard curves

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

1. Add 100 µL from each of the three reconstituted standard vials to a polypropylene tube and label as Std1.
2. Add 150 µL Assay Diluent (for serum and plasma samples), **or** 150 µL of a 50/50 diluent mixture (for other sample types) to each of 7 tubes. The last tube is to be used as a blank (for background determination).

   Note: To extend low-end quantitation, up to three additional tubes can be added to the standard curve (e.g., Std8, Std9, Std10).
3. Add 75 µL from one tube to the next one to make 1:3 serial dilutions of the standard. Mix thoroughly and change pipettes tips between steps.



4. Discard any remaining reconstituted standard.

Washing guidelines

5. Incomplete washing adversely affects assay results. Perform all wash steps with the 1X Wash Solution.

Magnetic plate separator

6. Place the 96-well flat bottom plate containing beads and 200 µL of 1X Wash Solution onto the magnetic separator.
7. Allow the beads to settle for 30–60 seconds.
8. 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.
9. Separate the plate from the magnetic separator before adding wash solution or any reagent to the plate.

Assay procedure

Total assay time is 3.5 hours.

IMPORTANT! Always keep beads and plate protected from light.

Analyte capture

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 µL of 1X Antibody Beads into each well. Protect the plate from light once the beads have been added.
4. Wash assay wells twice with 200 µL of 1X Wash Solution. (see "Washing guidelines").
5. Add 25 µL of Incubation Buffer into each well.
6. Add 50 µL of diluted standards into standard wells.
7. Add 50 µL of your blank into blank/background wells.
8. Add 25 µL of Assay Diluent followed by 25 µL of sample in sample wells.
9. Cover the plate 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.

Analyte detection

10. *Prepare 1X Biotinylated Detector Antibody in a conical tube**:

    For a single assay well:
    - Add 50 µL of Biotin Diluent.
    - Add 5 µL of 10X Biotinylated Antibody.

    Scale volumes according to the number of assay wells needed.
11. Decant liquid and wash the wells twice with 200 µL 1X Wash Solution (see "Washing guidelines").
12. Add 50 µL 1X Biotinylated Detector Antibody to each well. Cover and incubate the plate for 1 hour at room temperature on an orbital plate shaker.
13. *Prepare 1X Streptavidin-RPE solution in a conical tube**:

    For a single assay well:
    - Add 50 µL of RPE-Diluent.
    - Add 5 µL of 10X Streptavidin-RPE (protect from light).

    Scale volumes according to the number of assay wells needed.
14. Decant liquid and wash the wells twice with 200 µL 1X Wash Solution.
15. Add 50 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.
16. Decant liquid and wash the wells 3 times with 200 µL 1X Wash Solution.

* Dilution factor is 1:11 for extra pipetting volume.

Assay reading

17. 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. Bring the plate back to room temperature on an orbital plate shaker the next day. Remove 1X Wash Solution from stored plates, and begin procedure starting from step 17.
18. Uncover the plate and insert the plate into the XY platform of the Luminex® 100/200™, FLEXMAP 3D®, or MAGPIX® instrument, and analyze the samples.

    Determine the concentration of samples from the standard curve using curve fitting software.