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*	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 (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	Refer to the lot specific technical data sheet included with kit		

*Volume can be adjusted during acquisition to optimize bead count

xPONENT® software results

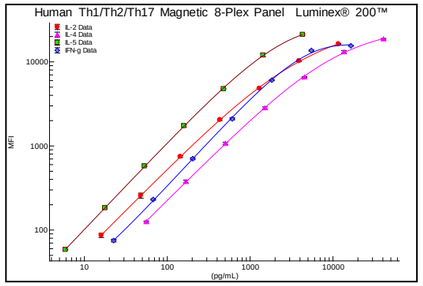
- Check bead counts to assure accurate data acquisition.
- Choose the individual analytes to view the standard curve and individual analyte statistics.
- Qualify all the standard points by checking for inaccurate standard points due to excessive plateauing or bottoming out. Net MFI-background and %Recovery limits (found in the statistics to the far right) are useful checks for the bottom and top of the curve. Use **Invalidate**, **Reanalyze** and **Save** to remove inaccurate data points (plateaus or bottom outs).
- Review %CV of Replicates (far right beside the %Recovery column). Note that %CV of Replicates calculation is based on concentration, not MFI data.
- Close results and use **Export CSV** to export to Excel readable file.

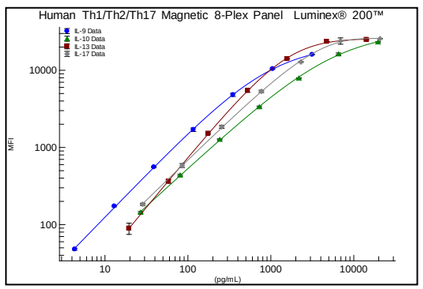
Performance characteristics

Cytokine	Bead region	Sensitivity (pg/mL)	Recovery (serum, EDTA plasma)	Recovery (citrate plasma, heparin plasma)	Recovery (TC, RPMI + 10% FBS)	Inter-assay precision
IL-2	54	<5	✓	✓	✓	1.6%
IL-4	77	<5	✓	✓	✓	2.0%
IL-5	34	<1	✓	✓	✓	3.6%
IL-9	44	<1	✓	✓	✓	1.9%
IL-10	15	<1	✓	✓	✓	2.0%
IL-13	18	<5	✓	✓	✓	1.8%
IL-17	25	<5	✓	✓	✓	2.1%
IFN-γ	38	<1	✓	–	✓	2.0%

(✓): 70–130% recovery, (–): 50–69% recovery, (+): 131–150% recovery, (NR-Not recommended): <50% or >150% recovery.

Typical standard curve





Note: The analysis results presented in the preceding table and figures were obtained during product development. Actual values may vary depending upon the lot being used to perform the assay.

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.lifetechnologies.com/termsandconditions.** If you have any questions, please contact Life Technologies **at www.lifetechnologies.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

novex by life technologies™

Human Th1/Th2/Th17 Magnetic 8-Plex Panel

Catalog no. LHC0015M

Quantity: 100 tests

Pub. Part no. MAN0007335 **Rev 1.00**

Description

The Human Th1/Th2/Th17 Magnetic 8-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 Th1/Th2/Th17 Magnetic 8-Plex Panel is designed for the quantitative determination of IL-2, IL-4, IL-5, IL-9, IL-10, IL-13, IL-17, and IFN-γ in serum, plasma, and tissue culture supernatant.

Note: The three samples types listed above have been validated but other sample types may be used.

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 Th1/Th2/Th17 8-Plex Magnetic Antibody Bead Concentrate (10X) (contains 0.05% sodium azide), light-sensitive	0.25 mL
Human Th1/Th2/Th17 8-Plex Magnetic Biotinylated Antibody Concentrate (10X) (contains 0.1% sodium azide)	1 mL
Human Cytokine 17-Plex Standard (contains 0.1% sodium azide)	2 vials
Wash Solution Concentrate (20X) (contains 0.1% sodium azide)	3 × 15 mL
Incubation Buffer	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)	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

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 and orbital shaker (small diameter rotation)
- Magnetic bead washing equipment (Magnetic 96-Well Separator, Cat. no. A14179 or equivalent, or Automated Plate Washer, Cat. No. 405TSRSLIFE or equivalent)
- Calibrated adjustable precision pipettes and glass or polypropylene tubes for diluting solutions
- Sonicating water bath

General Guidelines

- The complete assay is performed using the 96-well flat bottom plate provided in the kit. Although a 96-well filter plate might also be used in place of the 96-well flat bottom plate, it is not recommended because slow plate reading speed might occur.
- Do not invert the plate during the assay if using a filter plate.
- 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.

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

Manufacturing Site • 7335 Executive Way • Frederick • MD 21704 • E-mail: **techsupport@lifetech.com**

Before starting

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

Prepare wash solution

Prepare 1X Wash Solution by adding 1 part of Wash Solution Concentrate (20X) with 19 parts of deionized water. Mix well.

1X Wash Solution is stable for up to 2 weeks when stored at 2°C to 8°C.

Note: If there is precipitate in the Wash Solution Concentrate (20X), warm the bottle in a 37°C bath and mix until the precipitate is dissolved.

Prepare sample

If possible, avoid the use of hemolyzed or lipemic sera.

- Collect samples in pyrogen/endotoxin-free tubes. Centrifuge, separate, and transfer samples to polypropylene tubes for storage.
- Analyze samples shortly after collection or thawing. 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 (14,000 rpm for 10 minutes) or filter prior to analysis to prevent potential bead aggregation.
- In the event that the sample concentrations exceed the standard curve, dilute samples and reanalyze. Dilute the serum samples in Assay Diluent and dilute tissue culture supernatants in the corresponding tissue culture medium.

Reconstitute lyophilized standards

Each kit comes with two vials of standard, so that two runs on the plate can be made with freshly prepared standards.

- Reconstitute protein standard(s) within 1 hour of use.
- Perform standard dilutions in glass or polypropylene tubes.
- 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% mixture).
- **Do not vortex**. Avoid formation of foam when mixing or reconstituting protein solutions.

Reconstitute lyophilized standards, continued

For this 8-plex assay, mix and reconstitute one of the standard vials as follows:

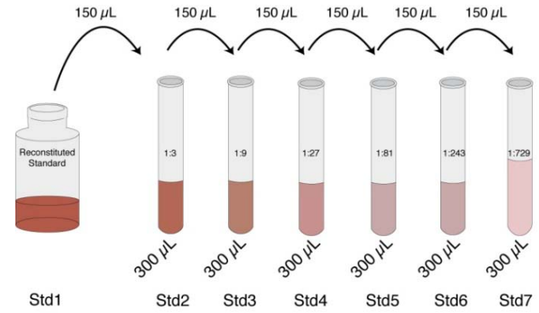
1. Add 1 mL of appropriate diluent to the vial and incubate for 10 minutes.
2. Gently mix the standard to ensure complete reconstitution, and incubate at room temperature for an additional 5 minutes.

Prepare standard curves

Perform a serial dilution of the reconstituted standard(s).

- Add 300 µL Assay Buffer to each of 6 tubes.
- Make 1:3 serial dilutions of the standard as described below in the dilution diagram. Mix thoroughly between steps.
- Discard any remaining reconstituted standard. Return the Assay Diluent to the refrigerator.

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



Assay procedure

Total assay time is 3.5 hours.

IMPORTANT! Perform a standard curve with each assay.

Note: Perform wash steps with a magnetic plate separator. Place the 96-well flat bottom plate with beads and 1X Wash Solution onto the magnetic separator. Allow the beads to settle for 30 seconds. Turn the magnetic separator and plate (held securely together) upside down, shake out the fluid, and blot excess liquid on dry paper towels. Separate the plate from the magnetic separator before adding wash solution or any reagent to the plate. Incomplete washing adversely affects assay results. Perform all wash steps with the 1X Wash Solution.

Analyte capture

1. Determine the number of wells in the 96-well plate to be used in the assay.
2. Immediately before use, vortex the 10X Antibody Beads for 30 seconds, then sonicate for 30 seconds.
3. *Prepare 1X Antibody Beads**: Add 2.5 µL of vortexed 10X Antibody Beads to 25 µL of 1X Wash Solution for each assay well. Each well requires 25 µL of 1X Antibody Beads.
4. Vortex the 1X Antibody Beads for 30 seconds prior to use in the assay. The magnetic beads settle rapidly. It is therefore important to mix well prior to use.
5. Add 25 µL of 1X Antibody Beads into designated wells. Keep the plate protected from light once the beads have been added.
6. Wash assay wells twice with 200 µL of 1X Wash Solution. Allow the beads to soak for 30 seconds before washing the plate (see note above for details).
7. Add 50 µL of Incubation Buffer into designated wells.
8. Add 100 µL of diluted standards into designated wells. Add 100 µL of Assay Diluent as a blank into designated wells. For wells designated for samples, add 50 µL of Assay Diluent followed by 50 µL of sample.
9. Cover and incubate the plate for 2 hours at room temperature on an orbital plate shaker (500–600 rpm).

   Note: Cover the assay plate using the plate cover provided with the kit during all incubations to avoid exposure to light. The speed may need to be adjusted depending upon the radius of the orbital shaker.

Analyte detection

10. *Prepare 1X Biotinylated Detector Antibody**: Add 10 µL of 10X Biotinylated Antibody to 100 µL of Biotin Diluent for each assay well. Each well requires 100 µL of 1X Biotinylated Antibody.
11. Wash assay wells twice with 200 µL of 1X Wash Solution.
12. Add 100 µL 1X Biotinylated Antibody to each assay well. Cover and incubate the plate for 1 hour at room temperature on an orbital plate shaker (500–600 rpm).
13. *Prepare 1X Streptavidin-RPE solution**: Add 10 µL of 10X Streptavidin-RPE to 100 µL of RPE-Diluent for each assay well (protect solution from light exposure). Each well requires 100 µL of the 1X Streptavidin-RPE solution.
14. Wash assay wells twice with 200 µL of 1X Wash Solution.
15. 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 (500–600 rpm).
16. Wash assay wells three times with 200 µL of 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 (500–600 rpm) 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 overnight at 2°C to 8°C for reading the following day without significant loss of fluorescent intensity. Remove 1X Wash Solution from stored plates and add 150 µL fresh 1X Wash Solution. Place the plate on an orbital plate shaker (500–600 rpm) for 2–3 minutes prior to analysis.
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.