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.

Important licensing information

This product may be covered by one or more Limited Use Label Licenses. By use of this product, you accept the terms and conditions of all applicable Limited Use Label Licenses.

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 Ultrasensitive Magnetic Buffer Reagent Kit

Catalog no. LHB0003M **Quantity:** 100 tests

Pub. No. MAN0010874 **Rev** 2.00

Description

The Human Ultrasensitive Magnetic Buffer Reagent Kit contains a set of common reagents for use with Human Ultrasensitive Magnetic Bead Kits and MAGPIX®, Luminex® 100™, 200™, or FLEXMAP 3D® systems. The xPONENT® software package is recommended for data analysis. To find out more, contact Technical Support at techsupport@lifetech.com.

The Human Ultrasensitive Magnetic Buffer Reagent Kit is designed for the quantitative determination of multiple extracellular proteins in human serum, plasma, and tissue culture supernatant.

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**.

Reagents Provided	Quantity
Wash Solution Concentrate (20X) (contains 0.1% sodium azide)	3 × 15 mL
Ultrasensitive Standard Reconstituiton Buffer (contains 0.1% sodium azide)	3 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	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

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)
- Handheld magnet such as the Magnetic 96-Well Separator (Cat. no. A14179)
- Calibrated adjustable precision pipettes and glass or plastic tubes for diluting solutions
- Sonicating water bath

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 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 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 15 mL of Wash Solution Concentrate (20X) to 285 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

- 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.
- 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

1. Prepare a 1:2 dilution of serum or plasma sample in Assay Diluent. For tissue culture supernatants, a minimum of 1:2 dilution (equal parts) with Assay Diluent is recommended.
2. 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 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 **polypropylene tubes**.
- Once reconstituted, standards **cannot** be stored for future use.
- **Do not vortex**. Avoid formation of foam when mixing or reconstituting protein solutions.

Reconstitute lyophilized standards, continued

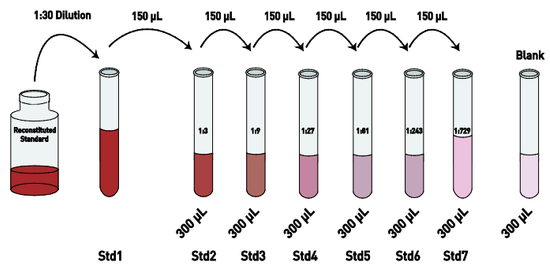
Mix and reconstitute one of the standard vials as follows:

1. Add the recommended volume of Ultrasensitive Standard Reconstitution Buffer to 1 standard vial and incubate for 10 minutes. Refer to the lot specific technical data sheet included with this kit for full reconstitution and dilution instructions.
2. Gently mix the standard to ensure complete reconstitution, and incubate at room temperature for an additional 5 minutes.
3. Perform a 1:30 dilution of the reconstituted standard before preparing the serial dilutions for the standard curve.
   - When working with serum or plasma samples, perform the 1:30 dilution in Ultrasensitive Assay Diluent.
   - For other sample types (e.g., tissue culture supernatants) perform the 1:30 dilution in a solution consisting of 50% Ultrasensitive Assay Diluent and 50% sample matrix.

Prepare standard curves

Run a standard curve with each assay. Perform a serial dilution of the **diluted** 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. The last tube is to be used as a blank (for background determination).
2. Add 150 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.



3. Discard any remaining reconstituted standard.

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

Washing guidelines

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

Magnetic plate separator

1. Place the 96-well flat bottom plate containing beads and 200 µL of 1X Wash Solution onto the magnetic separator.
2. Allow the beads to settle for 30–60 seconds.
3. 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.
   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! Perform a standard curve with each assay.

Analyte capture

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**:

   For a single assay well:
   - Add 25 µL of 1X Wash Solution.
   - Add 2.5 µL of vortexed Antibody Bead Concentrate (10X).

   Scale volumes according to the number of assay wells needed.
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 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 "Washing guidelines").
7. Add 50 µL Incubation Buffer to all assay wells.
8. Add 100 µL of diluted standards into standard wells.
9. Add 100 µL of your blank into blank/background wells.
10. Add 50 µL of Assay Diluent followed by 50 µL of sample in sample wells.
11. Cover and incubate the plate for 2 hours at room temperature 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

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

    For a single assay well:
    - Add 100 µL of Biotin Diluent.
    - Add 10 µL of 10X Biotinylated Antibody.

    Scale volumes according to the number of assay wells needed.
13. Decant liquid and wash the wells twice with 200 µL 1X Wash Solution (see "Washing guidelines").
14. Add 100 µL 1X Biotinylated Detector Antibody to each well. Cover and incubate the plate for 1 hour at room temperature on an orbital plate shaker.
15. *Prepare 1X Streptavidin-RPE solution in a conical tube*:*

    For a single assay well:
    - Add 100 µL of RPE-Diluent.
    - Add 10 µL of 10X Streptavidin-RPE (protect from light).

    Scale volumes according to the number of assay wells needed.
16. Decant liquid and wash the wells twice with 200 µL 1X Wash Solution.
17. 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.
18. 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

19. Add 150 µL 1X Wash Solution to each assay well and place the plate on the plate shaker for 2–3 minutes.

    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. To proceed after storage, decant 1X Wash Solution from stored plates, and begin procedure starting from step 18.
20. 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.