Human Kidney Toxicity
Magnetic Bead Panel 2

96 Well Plate Assay

Cat. # HKTX2MAG-38K
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For Research Use Only. Not for Use in Diagnostic Procedures.

By purchasing this product, which contains fluorescently labeled microsphere beads authorized by Luminex Corporation (“Luminex”), you, the customer, acquire the right under Luminex’s patent rights, if any, to use this product or any portion of this product, including without limitation the microsphere beads contained herein, only with Luminex’s laser based fluorescent analytical test instrumentation marketed under the name of Luminex 100™ IS, 200™, HTS, FLEXMAP 3D®, MAGPIX®.
INTRODUCTION

Absorption, distribution, metabolism and excretion (ADME) parameters are critical to all stages of a fully integrated drug development program and are used to augment the interpretation of toxicological findings. As toxicity has been found to be the leading cause of drug failure, this area of research is expanding in search of more sensitive, rapid methods for determining organ-specific damage as quickly as possible. Drug induced damage to kidney cells, also known as renal toxicity, results from drug excretion. The traditional methodology for determining renal toxicity has been to measure the blood urea nitrogen (BUN) and serum creatinine. These two tests only detect kidney damage a week after it begins to occur and only show that damage has occurred somewhere in the kidney. With EMD Millipore’s MILLIPLEX® MAP Human Kidney Toxicity Panels coupled with the Luminex xMAP® platform, you receive the advantage of speed and consistency, allowing quantitative multiplex detection of multiple analytes simultaneously, dramatically improving productivity.

Magnetic Beads can make the process of automation and high throughput screening easier with features such as walk-away washing. Advantages even outside automation include:

- More flexible plate and plate washer options
- Improved performance with turbid serum samples
- Automated washing eliminates technical obstacles (i.e. clogging of wells that contain viscous samples) which may result during vacuum manifold/manual washing

Coupled with the Luminex xMAP® platform in a magnetic bead format, you receive the advantage of ideal speed and sensitivity, allowing quantitative multiplex detection of dozens of analytes simultaneously, which can dramatically improve productivity.

EMD Millipore’s MILLIPLEX® MAP Human Kidney Toxicity Panels are the most versatile system available for nephrotoxicity research.

- MILLIPLEX® MAP offers you the ability to:
  - Choose any combination of analytes from our panel of 3 analytes to design a custom kit that better meets your needs.
  - A convenient “all-in-one” box format gives you the assurance that you will have all the necessary reagents you need to run your assay.

EMD Millipore’s MILLIPLEX® MAP Human Kidney Toxicity Panel 2 kit is to be used for the simultaneous quantification of the following 3 human kidney toxicity biomarkers in any combination in serum: β-2-Microglobulin, Clusterin and Cystatin C.

This kit may be used for the analysis of all or any combination of the above human kidney toxicity biomarkers in serum samples.

*For Research Use Only. Not for Use in Diagnostic Procedures.*

*Please read entire protocol before use.*

*It is important to use same assay incubation conditions throughout your study.*
PRINCIPLE

MILLIPLEX® MAP is based on the Luminex xMAP® technology — one of the fastest growing and most respected multiplex technologies offering applications throughout the life-sciences and capable of performing a variety of bioassays including immunoassays on the surface of fluorescent-coded magnetic beads known as MagPlex®-C microspheres.

- Luminex uses proprietary techniques to internally color-code microspheres with two fluorescent dyes. Through precise concentrations of these dyes, distinctly colored bead sets of 500 5.6 µm polystyrene microspheres or 80 6.45 µm magnetic microspheres can be created, each of which is coated with a specific capture antibody.
- After an analyte from a test sample is captured by the bead, a biotinylated detection antibody is introduced.
- The reaction mixture is then incubated with Streptavidin-PE conjugate, the reporter molecule, to complete the reaction on the surface of each microsphere.
- EMD Millipore provides three Luminex instruments to acquire and analyze data using two detection methods:
  - The Luminex analyzers Luminex 200™ and FLEXMAP 3D®, flow cytometry-based instruments that integrate key xMAP® detection components, such as lasers, optics, advanced fluidics and high-speed digital signal processors.
  - The Luminex analyzer (MAGPIX®), a CCD-based instrument that integrates key xMAP® capture and detection components with the speed and efficiency of magnetic beads.
- Each individual microsphere is identified and the result of its bioassay is quantified based on fluorescent reporter signals. EMD Millipore combines the streamlined data acquisition power of Luminex xPONENT® acquisition software with sophisticated analysis capabilities of the new MILLIPLEX® Analyst 5.1, integrating data acquisition and analysis seamlessly with all Luminex instruments.

The capability of adding multiple conjugated beads to each sample results in the ability to obtain multiple results from each sample. Open-architecture xMAP® technology enables multiplexing of many types of bioassays reducing time, labor and costs over traditional methods.

STORAGE CONDITIONS UPON RECEIPT

- Recommended storage for kit components is 2 - 8°C.
- For long-term storage, freeze reconstituted standards and controls at ≤ -20°C. Avoid multiple (>2) freeze/thaw cycles.

DO NOT FREEZE Antibody-Immobilized Beads, Detection Antibody, and Streptavidin-Phycoerythrin.
REAGENTS SUPPLIED

Note: Store all reagents at 2 – 8°C

<table>
<thead>
<tr>
<th>REAGENTS SUPPLIED</th>
<th>CATALOG NUMBER</th>
<th>VOLUME</th>
<th>QUANTITY</th>
</tr>
</thead>
<tbody>
<tr>
<td>Human Kidney Toxicity Panel Standard</td>
<td>HKTX-8038</td>
<td>lyophilized</td>
<td>1 vial</td>
</tr>
<tr>
<td>Human Kidney Toxicity Panel Quality Controls 1 and 2</td>
<td>HKTX-6038</td>
<td>lyophilized</td>
<td>2 vials</td>
</tr>
<tr>
<td>Set of one 96-Well Plate with 2 Sealers</td>
<td>---------------</td>
<td>------------</td>
<td>----------</td>
</tr>
<tr>
<td>Assay Buffer</td>
<td>L-AB1</td>
<td>30 mL</td>
<td>2 bottles</td>
</tr>
<tr>
<td>10X Wash Buffer</td>
<td>L-WB</td>
<td>30 mL</td>
<td>2 bottles</td>
</tr>
<tr>
<td>Human Kidney Toxicity Panel Detection Antibodies</td>
<td>HKTX-1038</td>
<td>5.5 mL</td>
<td>1 bottle</td>
</tr>
<tr>
<td>Streptavidin-Phycoerythrin</td>
<td>L-SAPE</td>
<td>5.5 mL</td>
<td>1 bottle</td>
</tr>
<tr>
<td>Bead Diluent</td>
<td>LBD-3</td>
<td>3.5 mL</td>
<td>1 bottle</td>
</tr>
<tr>
<td>Mixing Bottle</td>
<td>---------------</td>
<td>------------</td>
<td>----------</td>
</tr>
</tbody>
</table>

**Human Kidney Toxicity Panel 2 Antibody-Immobilized Magnetic Beads:**

<table>
<thead>
<tr>
<th>Bead/Analyte Name</th>
<th>Luminex Magnetic Bead Region</th>
<th>Customizable Beads (20X Concentration, 200 µL)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Available</td>
<td>Cat. #</td>
</tr>
<tr>
<td>Anti – β-2-Microglobulin</td>
<td>36</td>
<td>✔ HB2MG-MAG</td>
</tr>
<tr>
<td>Anti – Clusterin</td>
<td>47</td>
<td>✔ HCLSTRN-MAG</td>
</tr>
<tr>
<td>Anti – Cystatin C</td>
<td>56</td>
<td>✔ HCYSTNC-MAG</td>
</tr>
</tbody>
</table>
MATERIALS REQUIRED BUT NOT PROVIDED

Reagents
1. Luminex Sheath Fluid (Luminex Catalog #40-50000) or Luminex Drive Fluid (Luminex Catalog # MPXDF-4PK)

Instrumentation / Materials
1. Adjustable Pipettes with Tips capable of delivering 25 µL to 1000 µL
2. Multichannel Pipettes capable of delivering 5 µL to 50 µL or 25 µL to 200 µL
3. Reagent Reservoirs
4. Polypropylene Microfuge Tubes
5. Rubber Bands
6. Aluminum Foil
7. Absorbent Pads
8. Laboratory Vortex Mixer
9. Sonicator (Branson Ultrasonic Cleaner Model #B200 or equivalent)
10. Titer Plate Shaker (Lab-Line Instruments Model #4625 or equivalent)
11. Luminex 200™, HTS, FLEXMAP 3D™, or MAGPIX® with xPONENT® software by Luminex Corporation
12. Automatic Plate Washer for magnetic beads (BioTek® 405 LS and 405 TS, EMD Millipore Catalog #40-094, #40-095, #40-096, #40-097 or equivalent) or Handheld Magnetic Separation Block (EMD Millipore Catalog #40-285 or equivalent).

Note: If a plate washer or handheld magnetic separation block for magnetic beads is not available, one can use a microtiter filter plate (EMD Millipore Catalog #MX-PLATE) to run the assay using a Vacuum Filtration Unit (EMD Millipore Vacuum Manifold Catalog #MSVMHTS00 or equivalent with EMD Millipore Vacuum Pump Catalog #WP6111560 or equivalent).

SAFETY PRECAUTIONS

- All blood components and biological materials should be handled as potentially hazardous. Follow universal precautions as established by the Centers for Disease Control and Prevention and by the Occupational Safety and Health Administration when handling and disposing of infectious agents.
- Sodium Azide or Proclin has been added to some reagents as a preservative. Although the concentrations are low, Sodium Azide and Proclin may react with lead and copper plumbing to form highly explosive metal azides. Dispose of unused contents and waste in accordance with international, federal, state, and local regulations.
TECHNICAL GUIDELINES

To obtain reliable and reproducible results, the operator should carefully read this entire manual and fully understand all aspects of each assay step before running the assay. The following notes should be reviewed and understood before the assay is set up.

- FOR RESEARCH USE ONLY. NOT FOR USE IN DIAGNOSTIC PROCEDURES.
- Do not use beyond the expiration date on the label.
- Do not mix or substitute reagents with those from other lots or sources.
- The Antibody-Immobilized Beads are light sensitive and must be protected from light at all times. Cover the assay plate containing beads with opaque plate lid or aluminum foil during all incubation steps.
- It is important to allow all reagents to warm to room temperature (20-25°C) before use in the assay.
- Incomplete washing can adversely affect the assay outcome. All washing must be performed with the Wash Buffer provided.
- The standards prepared by serial dilution must be used within 1 hour of preparation. Discard any unused standards except the standard stock which may be stored at ≤ -20°C for 1 month and at ≤ -80°C for greater than one month.
- If samples fall outside the dynamic range of the assay, further dilute the samples with the appropriate diluent and repeat the assay.
- Any unused mixed Antibody-Immobilized Beads may be stored in the Mixing Bottle at 2-8°C for up to one month.
- During the preparation of the standard curve, make certain to mix the higher concentration well before making the next dilution. Use a new tip with each dilution.
- The plate should be read immediately after the assay is finished. If, however, the plate cannot be read immediately, seal the plate, cover with aluminum foil or an opaque lid, and store the plate at 2-8°C for up to 24 hours. Prior to reading, agitate the plate on the plate shaker at room temperature for 10 minutes. Delay in reading a plate may result in decreased sensitivity for some analytes.
- The titer plate shaker should be set at a speed to provide maximum orbital mixing without splashing of liquid outside the wells. For the recommended plate shaker, this would be a setting of 5-7 which is approximately 500-800 rpm.
- Ensure that the needle probe is clean. This may be achieved by sonication and/or alcohol flushes.
- When reading the assay on Luminex 200™, adjust probe height according to the protocols recommended by Luminex to the kit solid plate or to the recommended EMD Millipore filter plates using 3 alignment discs. When reading the assay on MAGPIX®, adjust probe height according to the protocols recommended by Luminex to the kit solid plate or to the recommended EMD Millipore filter plates using 2 alignment discs. When reading the assay on FLEXMAP 3D®, adjust probe height according to the protocols recommended by Luminex to the kit solid plate using 1 alignment disc. For FLEXMAP 3D® when using the solid plate in the kit, the final resuspension should be with 150 μL Sheath Fluid in each well and 75 μL should be aspirated.
- For cell culture supernatants or tissue extraction, use the culture or extraction medium as the matrix solution in background, standard curve and control wells. If samples are diluted in assay buffer, use the assay buffer as matrix.
TECHNICAL GUIDELINES (continued)

- For cell/tissue homogenate, the final cell or tissue homogenate should be prepared in a buffer that has a neutral pH, contains minimal detergents or strong denaturing detergents, and has an ionic strength close to physiological concentration. Avoid debris, lipids, and cell/tissue aggregates. Centrifuge samples before use.
- Vortex all reagents well before adding to plate.

TECHNICAL GUIDELINES (continued)

- Ensure that the needle probe is clean. This may be achieved by sonication and/or alcohol flushes.
- For serum samples, use the Assay Buffer provided in this kit as the matrix solution for background, standard, and quality control wells.
- Vortex all reagents well before adding to plate.

SAMPLE COLLECTION AND STORAGE

A. Preparation of Serum Samples:
   - Allow the blood to clot for 30 minutes at room temperature then centrifuge the samples for 10 minutes at 1000 x g. Finally, collect the serum samples and use them immediately in the assay or aliquot and store them at ≤ -20°C.
   - Avoid multiple (>2) freeze/thaw cycles.
   - When using frozen samples, it is recommended to thaw the samples completely, mix well by vortexing and centrifuge prior to use in the assay to remove particulates.
   - Customers need to determine the optimal dilution factor for their samples. Generally, serum samples from normal subjects should be diluted 1:2000 using the Assay Buffer provided in the kit as the sample diluent (i.e. Add 5 µL sample to 95 µL Assay Buffer then add 5 µL diluted sample to 495 µL Assay Buffer). If samples require dilution beyond 1:2000, continue to use Assay Buffer as the sample diluent.
   - For data analysis, multiply the final concentration of each sample by the dilution factor.

NOTE:
- A maximum of 25 µL per well of diluted serum sample can be used.
- All samples must be stored in polypropylene tubes. **DO NOT STORE SAMPLES IN GLASS.**
- Avoid debris, lipids and cells when using samples with gross hemolysis or lipemia.
A. Preparation of Antibody-Immobilized Beads

Sonicate each individual antibody-bead vial for 30 seconds then vortex for 1 minute. Add 150 µL from each antibody-bead vial to the Mixing Bottle and bring final volume to 3.0 mL with Bead Diluent. Vortex the mixed beads well. Unused portion may be stored at 2-8°C for up to one month. (Note: Due to the composition of magnetic beads, you may notice a slight color in the bead solution. This does not affect the performance of the beads or the kit.)

Example 1: When using 1 antibody-immobilized bead, add 150 µL from the bead vial to the Mixing Bottle. Then add 2.85 mL Bead Diluent.

Example 2: When using 3 antibody-immobilized beads, add 150 µL from each of the 3 bead vials to the Mixing Bottle. Then add 2.55 mL Bead Diluent.

B. Preparation of Quality Controls

Before use, reconstitute Quality Control 1 and Quality Control 2 with 250 µL deionized water. Invert the vial several times to mix then vortex briefly. Allow the vial to sit for 5-10 minutes and then transfer the controls to appropriately labeled polypropylene microfuge tubes. Unused portions may be stored at ≤-20°C for up to one month.

C. Preparation of Wash Buffer

Bring the 10X Wash Buffer to room temperature and mix to bring all salts into solution. Dilute 30 mL of 10X Wash Buffer with 270 mL deionized water. Store unused portion at 2-8°C for up to one month.

D. Preparation of Human Kidney Toxicity Panel Standard

1.) Prior to use, reconstitute the Human Kidney Toxicity Panel Standard with 250 µL deionized water (refer to table below for analyte concentrations). Invert the vial several times to mix. Vortex the vial for 10 seconds. Allow the vial to sit for 5-10 minutes and then transfer the standard to an appropriately labeled polypropylene microfuge tube. This will be used as Standard 6; the unused portion may be stored at ≤-20°C for up to one month.

2.) Preparation of Working Standards

Label five polypropylene microfuge tubes Standard 5, Standard 4, Standard 3, Standard 2 and Standard 1. Add 120 µL of Assay Buffer to each of the five tubes. Prepare serial dilutions by adding 40 µL of the reconstituted standard (Standard 6) to the Standard 5 tube, mix well and transfer 40 µL of the Standard 5 to the Standard 4 tube, mix well and transfer 40 µL of the Standard 4 to the Standard 3 tube, mix well and transfer 40 µL of the Standard 3 to the Standard 2 tube, mix well and transfer 40 µL of the Standard 2 to the Standard 1 tube and mix well. The 0 pg/mL Standard (Background) will be Assay Buffer.
### Standard (Tube #) | Volume of Deionized Water to Add | Volume of Standard to Add
--- | --- | ---
Standard 6 (reconstituted standard) | 250 µL | 0

| Standard (Tube #) | Volume of Assay Buffer to Add | Volume of Standard to Add |
| --- | --- | ---
| Standard 5 | 120 µL | 40 µL of Standard 6 |
| Standard 4 | 120 µL | 40 µL of Standard 5 |
| Standard 3 | 120 µL | 40 µL of Standard 4 |
| Standard 2 | 120 µL | 40 µL of Standard 3 |
| Standard 1 | 120 µL | 40 µL of Standard 2 |

### Preparation of Standards

![Diagram of standard preparation process]

After dilution, each tube has the following concentrations for each analyte:

<table>
<thead>
<tr>
<th>Standard</th>
<th>β-2-Microglobulin (pg/mL)</th>
<th>Cystatin C (pg/mL)</th>
<th>Clusterin (pg/mL)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Standard 1</td>
<td>49</td>
<td>9.8</td>
<td>1,953</td>
</tr>
<tr>
<td>Standard 2</td>
<td>195</td>
<td>39.1</td>
<td>7,813</td>
</tr>
<tr>
<td>Standard 3</td>
<td>781</td>
<td>156.3</td>
<td>31,250</td>
</tr>
<tr>
<td>Standard 4</td>
<td>3,125</td>
<td>625.0</td>
<td>125,000</td>
</tr>
<tr>
<td>Standard 5</td>
<td>12,500</td>
<td>2,500.0</td>
<td>500,000</td>
</tr>
<tr>
<td>Standard 6</td>
<td>50,000</td>
<td>10,000.0</td>
<td>2,000,000</td>
</tr>
</tbody>
</table>
IMMUNOASSAY PROCEDURE

- Prior to beginning this assay, it is imperative to read this protocol completely and to thoroughly understand the Technical Guidelines.
- Allow all reagents to warm to room temperature (20-25°C) before use in the assay.
- Diagram the placement of Background, Standards 1 through 6, Controls 1 and 2, and Samples on Well MAP Worksheet in a vertical configuration. (Note: Most instruments will only read the 96-well plate vertically by default.) It is recommended to run the samples in duplicate.
- If using a filter plate, set the filter plate on a plate holder at all times during reagent dispensing and incubation steps so the bottom of the plate does not touch any surface.

1. Add 200 µL of Assay Buffer into each well of the plate. Seal and mix on a plate shaker for 10 minutes at room temperature (20-25°C).
2. Decant Assay Buffer and remove the residual amount from all wells by inverting the plate and tapping it smartly onto absorbent towels several times.
3. Add 25 µL of Assay Buffer to Background wells.
4. Add 25 µL of each Standard or Control into the appropriate wells.
5. Add 25uL Assay Buffer to Background, Standard and Control wells.
6. Add 25 µL of Assay Buffer to sample wells.
7. Add 25 µL of 1:2000 diluted Sample into the appropriate wells.
8. Vortex Mixing Bottle and add 25 µL of the Mixed Beads to each well. (Note: During addition of Mixed Beads, shake Mixing Bottle intermittently to avoid settling.)
9. Seal the plate with a plate sealer. Wrap the plate with foil and incubate with agitation on a plate shaker overnight (16 – 18 hours) at 4°C.
10. Gently remove well contents and wash plate 2 times following instructions listed in the PLATE WASHING section.

11. Add 50 µL of Detection Antibodies into each well. (Note: Allow the Detection Antibodies to warm to room temperature prior to addition.)

12. Seal, cover with foil, and incubate with agitation on a plate shaker for 1 hour at room temperature (20-25°C). **DO NOT ASPIRATE AFTER INCUBATION.**

13. Add 50 µL Streptavidin-Phycoerythrin to each well containing the 50 µL of Detection Antibodies.

14. Seal, cover with foil and incubate with agitation on a plate shaker for 30 minutes at room temperature (20-25°C).

15. Gently remove well contents and wash plate 2 times following instructions listed in the PLATE WASHING section.

16. Add 100 µL of Sheath Fluid (or Drive Fluid if using MAGPIX®) to all wells. Resuspend the beads by incubating with agitation on a plate shaker for 5 minutes at room temperature.

17. Run plate on Luminex 200™, HTS or FLEXMAP 3D® or MAGPIX® with xPONENT® software.

18. Save and analyze the Median Fluorescent Intensity (MFI) data using a 5-parameter logistic or spline curve-fitting method to calculate analyte concentrations in diluted samples. Multiply calculated concentrations by 2000 to determine sample concentration.
PLATE WASHING
If using a solid plate, use either a handheld magnet or magnetic plate washer.

1.) Solid Plate
If using a solid plate, use either a handheld magnet or magnetic plate washer.
A.) Handheld magnet (EMD Millipore Catalog #40-285) - Rest plate on magnet for 60 seconds to allow complete settling of magnetic beads. Remove well contents by gently decanting the plate in an appropriate waste receptacle and gently tapping on absorbent pads to remove residual liquid. Wash plate with 200 µL of Wash Buffer by removing plate from magnet, adding Wash Buffer, shaking for 30 seconds, reattaching to magnet, letting beads settle for 60 seconds and removing well contents as previously described after each wash. Repeat wash steps as recommended in Assay Procedure.
B.) Magnetic plate washer (EMD Millipore Catalog #40-094, #40-095, #40-096 and #40-097) - Please refer to specific automatic plate washer manual for appropriate equipment settings. Please note that after the final aspiration, there will be approximately 25 µL of residual wash buffer in each well. This is expected when using the BioTek plate washer and this volume does not need to be aspirated from the plate.

If using an automatic plate washer other than BioTek® 405 LS or 405 TS, please refer to the manufacturer's recommendations for programming instructions.

2.) Filter Plate (EMD Millipore Catalog #MX-PLATE)
If using a filter plate, use a vacuum filtration manifold to remove well contents. Wash plate with 200 µL/well of Wash Buffer, removing Wash Buffer by vacuum filtration after each wash. Repeat wash steps as recommended in the Assay Procedure.

EQUIPMENT SETTINGS
Luminex 200™, HTS, FLEXMAP 3D®, and MAGPIX® with xPONENT® software:

These specifications are for the Luminex 200™, Luminex HTS, Luminex FLEXMAP 3D®, and Luminex MAGPIX® with xPONENT® software. Luminex instruments with other software (e.g. MasterPlex®, STarStation, LiquiChip, Bio-Plex Manager™, LABScan™ 100) would need to follow instrument instructions for gate settings and additional specifications from the vendors for reading Luminex magnetic beads.

For magnetic bead assays, the Luminex 200™ and HTS instruments must be calibrated with the xPONENT® 3.1 compatible Calibration Kit (EMD Millipore Catalog #40-275) and performance verified with the Performance Verification Kit (EMD Millipore Catalog #40-276). The Luminex FLEXMAP 3D® instrument must be calibrated with the FLEXMAP 3D® Calibrator Kit (EMD Millipore Catalog #40-028) and performance verified with the FLEXMAP 3D® Performance Verification Kit (EMD Millipore Catalog #40-029). The Luminex MAGPIX® instrument must be calibrated with the MAGPIX® Calibration Kit (EMD Millipore Catalog #40-049) and performance verified with the MAGPIX® Performance Verification Kit (EMD Millipore Catalog #40-050).

NOTE: When setting up a Protocol using the xPONENT® software, you must select MagPlex as the Bead Type in the Acquisition settings.
NOTE: These assays cannot be run on any instruments using Luminex IS 2.3 or Luminex 1.7 software.
EQUIPMENT SETTINGS (continued)

The Luminex probe height must be adjusted to the plate provided in the kit. Please use Catalog #MAG-PLATE, if additional plates are required for this purpose.

<table>
<thead>
<tr>
<th>Events:</th>
<th>50 per bead</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sample Size:</td>
<td>50 µL</td>
</tr>
<tr>
<td>Gate Settings</td>
<td>8,000 to 15,000</td>
</tr>
<tr>
<td>Time Out:</td>
<td>60 seconds</td>
</tr>
<tr>
<td>Bead Set:</td>
<td>3-plex Bead Regions</td>
</tr>
<tr>
<td></td>
<td>β-2-Microglobulin 36</td>
</tr>
<tr>
<td></td>
<td>Clusterin 47</td>
</tr>
<tr>
<td></td>
<td>Cystatin C 56</td>
</tr>
</tbody>
</table>

QUALITY CONTROLS

The ranges for each analyte in Quality Control 1 and 2 are provided on the card insert or can be located at the EMD Millipore Corporation website www.millipore.com/techlibrary/index.do using the catalog number as the keyword.

ASSAY CHARACTERISTICS

Cross-Reactivity

There was no or negligible cross-reactivity between the antibodies for an analyte and any of the other analytes in this panel.
Assay Sensitivities (minimum detectable concentrations, pg/mL)

Minimum Detectable Concentration (MinDC) is calculated using the MILLIPLEX® Analyst Software from EMD Millipore. It measures the true limits of detection for an assay by mathematically determining what the empirical MinDC would be if an infinite number of standard concentrations were run for the assay under the same conditions in an overnight assay.

<table>
<thead>
<tr>
<th>Analyte</th>
<th>MinDC + 2SD (pg/ml)</th>
</tr>
</thead>
<tbody>
<tr>
<td>β-2-Microglobulin</td>
<td>57</td>
</tr>
<tr>
<td>Clusterin</td>
<td>930</td>
</tr>
<tr>
<td>Cystatin C</td>
<td>149</td>
</tr>
</tbody>
</table>

N=9 assays

Precision

Intra-assay precision is generated from the mean of the %CV's from 8 reportable results across two different concentrations of analytes in a single assays. Inter-assay precision is generated from the mean of the %CV’s across two different concentrations of analytes across six different assays.

<table>
<thead>
<tr>
<th>Analyte</th>
<th>Intra-Assay (%CV)</th>
<th>Inter-Assay (%CV)</th>
</tr>
</thead>
<tbody>
<tr>
<td>β-2-Microglobulin</td>
<td>16</td>
<td>16</td>
</tr>
<tr>
<td>Clusterin</td>
<td>7</td>
<td>3</td>
</tr>
<tr>
<td>Cystatin C</td>
<td>9</td>
<td>7</td>
</tr>
</tbody>
</table>

Accuracy

Spike Recovery: The data represent mean percent recovery of 3 levels of spiked standards in serum samples (n=5).

<table>
<thead>
<tr>
<th>Analyte</th>
<th>Spike Recovery in Serum</th>
</tr>
</thead>
<tbody>
<tr>
<td>β-2-Microglobulin</td>
<td>98</td>
</tr>
<tr>
<td>Clusterin</td>
<td>106</td>
</tr>
<tr>
<td>Cystatin C</td>
<td>102</td>
</tr>
</tbody>
</table>
### TROUBLESHOOTING GUIDE

<table>
<thead>
<tr>
<th>Problem</th>
<th>Probable Cause</th>
<th>Solution</th>
</tr>
</thead>
<tbody>
<tr>
<td>Insufficient bead count</td>
<td>Plate washer aspirate height set too low</td>
<td>Adjust aspiration height according to manufacturers’ instructions.</td>
</tr>
<tr>
<td></td>
<td>Bead mix prepared inappropriately</td>
<td>Sonicate bead vials and vortex just prior to adding to bead mix bottle according to protocol. Agitate bead mix intermittently in reservoir while pipetting this into the plate.</td>
</tr>
<tr>
<td></td>
<td>Samples interference due to particulate matter or viscosity</td>
<td>See above. Also sample probe may need to be cleaned with alcohol flushes, back flushes and washes; or, if needed, probe should be removed and sonicated.</td>
</tr>
<tr>
<td></td>
<td>Probe height not adjusted correctly</td>
<td>When reading the assay on Luminex 200™, adjust probe height to the kit solid plate or to the recommended EMD Millipore filter plates using 3 alignment discs. When reading the assay on MAGPIX®, adjust probe height to the kit solid plate or to the recommended EMD Millipore filter plates using 2 alignment discs. When reading the assay on FLEXMAP 3D®, adjust probe height to the kit solid plate using 1 alignment disc. For FLEXMAP 3D® when using the solid plate in the kit, the final resuspension should be with 150 μL Sheath Fluid in each well and 75 μL should be aspirated.</td>
</tr>
<tr>
<td>Background is too high</td>
<td>Background wells were contaminated</td>
<td>Avoid cross-well contamination by using sealer appropriately and pipetting with multichannel pipettes without touching reagent in plate.</td>
</tr>
<tr>
<td></td>
<td>Matrix used has endogenous analyte or interference</td>
<td>Check matrix ingredients for cross-reacting components (e.g. interleukin modified tissue culture medium).</td>
</tr>
<tr>
<td></td>
<td>Insufficient washes</td>
<td>Increase number of washes.</td>
</tr>
<tr>
<td>Beads not in region or gate</td>
<td>Luminex instrument not calibrated correctly or recently</td>
<td>Calibrate Luminex instrument based on manufacturer’s instructions, at least once a week or if temperature has changed by &gt;3°C.</td>
</tr>
<tr>
<td></td>
<td>Gate settings not adjusted correctly</td>
<td>Some Luminex instruments (e.g. Bio-Plex®) require different gate settings than those described in the kit protocol. Use instrument default settings.</td>
</tr>
<tr>
<td></td>
<td>Wrong bead regions in protocol template</td>
<td>Check kit protocol for correct bead regions or analyte selection.</td>
</tr>
<tr>
<td></td>
<td>Incorrect sample type used</td>
<td>Samples containing organic solvents or if highly viscous should be diluted or dialyzed as required.</td>
</tr>
<tr>
<td></td>
<td>Instrument not washed or primed</td>
<td>Prime the Luminex instrument 4 times to rid of air bubbles, wash 4 times with sheath fluid or water if there is any remnant alcohol or sanitizing liquid.</td>
</tr>
<tr>
<td></td>
<td>Beads were exposed to</td>
<td>Keep plate and bead mix covered with dark</td>
</tr>
<tr>
<td>Light</td>
<td>Lid or aluminum foil during all incubation steps.</td>
<td></td>
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<tr>
<td>---</td>
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</tr>
</tbody>
</table>
| Signal for whole plate is same as background | Incorrect or no Detection Antibody was added  
Streptavidin-Phycoerythrin was not added | Add appropriate Detection Antibody and continue.  
Add Streptavidin-Phycoerythrin according to protocol. If Detection Antibody has already been removed, sensitivity may be low. |
| Low signal for standard curve | Detection Antibody may have been removed prior to adding Streptavidin Phycoerythrin  
Incubations done at inappropriate temperatures, timings or agitation | May need to repeat assay if desired sensitivity not achieved.  
Assay conditions need to be checked. |
| Signals too high, standard curves are saturated | Calibration target value set too high  
Plate incubation was too long with standard curve and samples | With some Luminex instruments (e.g. Bio-Plex®) default target setting for RP1 calibrator is set at high PMT. Use low target value for calibration and reanalyze plate.  
Use shorter incubation time. |
| Sample readings are out of range | Samples contain no or below detectable levels of analyte  
Samples contain analyte concentrations higher than highest standard point  
Standard curve was saturated at higher end of curve | If below detectable levels, it may be possible to use higher sample volume. Check with technical support for appropriate protocol modifications.  
Samples may require dilution and reanalysis for just that particular analyte.  
See above. |
| High variation in samples and/or standards | Multichannel pipette may not be calibrated  
Plate washing was not uniform  
Samples may have high particulate matter or other interfering substances  
Plate agitation was insufficient  
Cross-well contamination | Calibrate pipettes.  
Confirm all reagents are removed completely in all wash steps.  
See above.  
Plate should be agitated during all incubation steps using an orbital plate shaker at a speed where beads are in constant motion without causing splashing.  
Check when reusing plate sealer that no reagent has touched sealer. Care should be taken when using same pipette tips that are used for reagent additions and that pipette tip does not touch reagent in plate. |
| FOR FILTER PLATES ONLY | Filter plate will not vacuum  
Vacuum pressure is insufficient | Increase vacuum pressure such that 0.2mL buffer can be suctioned in 3-5 seconds. |
Samples have insoluble particles | Centrifuge samples just prior to assay set-up and use supernatant.
---|---
High lipid concentration | After centrifugation, remove lipid layer and use supernatant.

<table>
<thead>
<tr>
<th>Plate leaked</th>
<th>Vacuum pressure too high</th>
</tr>
</thead>
<tbody>
<tr>
<td>Plate set directly on table or absorbent towels during incubations or reagent additions</td>
<td>Adjust vacuum pressure such that 0.2mL buffer can be suctioned in 3-5 seconds. May need to transfer contents to a new (blocked) plate and continue.</td>
</tr>
<tr>
<td>Insufficient blotting of filter plate bottom causing wicking</td>
<td>Set plate on plate holder or raised edge so bottom of filter is not touching any surface.</td>
</tr>
<tr>
<td>Pipette touching plate filter during additions</td>
<td>Blot the bottom of the filter plate well with absorbent towels after each wash step.</td>
</tr>
<tr>
<td>Probe height not adjusted correctly</td>
<td>Pipette to the side of plate.</td>
</tr>
<tr>
<td>Sample too viscous</td>
<td>Adjust probe to 3 alignment discs in well H6.</td>
</tr>
</tbody>
</table>

**REPLACEMENT REAGENTS**

**Components**

| Human Kidney Toxicity Panel Standard | HKTX-8038 |
| Human Kidney Toxicity Panel Quality Controls 1 and 2 | HKTX-6038 |
| Human Kidney Toxicity Panel Detection Antibodies | HKTX-1038 |
| Streptavidin-Phycoerythrin | L-SAPE |
| Assay Buffer | L-AB1 |
| Bead Diluent | LBD-3 |
| Set of two 96-Well Plates with Sealers | MAG-PLATE |
| 10X Wash Buffer | L-WB |

**Antibody-Immobilized Magnetic Beads**

<table>
<thead>
<tr>
<th>Kidney Toxicity Biomarker</th>
<th>Bead #</th>
<th>Cat. #</th>
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<tbody>
<tr>
<td>β-2-Microglobulin</td>
<td>36</td>
<td>HB2MG-MAG</td>
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<tr>
<td>Clusterin</td>
<td>47</td>
<td>HCLSTRN-MAG</td>
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<tr>
<td>Cystatin C</td>
<td>56</td>
<td>HCYSTNC-MAG</td>
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</table>
ORDERING INFORMATION

To place an order:

To assure the clarity of your custom kit order, please FAX the following information to our customer service department:

Include:
• Your name, telephone and/or fax number
• Customer account number
• Shipping and billing address
• Purchase order number
• Catalog number and description of product
• Quantity of kits
• Selection of MILLIPLEX® Analytes

FAX: (636) 441-8050

Toll-Free US: (866) 441-8400
(636) 441-8400

Mail Orders: EMD Millipore Corporation
6 Research Park Drive
St. Charles, Missouri 63304 U.S.A.

For International Customers:

To best serve our international customers in placing an order or obtaining additional information about MILLIPLEX® MAP products, please contact your multiplex specialist or sales representative or email our European Customer Service at customerserviceEU@merckgroup.com.

Conditions of Sale

For Research Use Only. Not for Use in Diagnostic Procedures.

Material Safety Data Sheets (MSDS)

Material Safety Data Sheets for EMD Millipore products may be ordered by fax or phone or through our website at www.emdmillipore.com/techlibrary/index.do.

Technical Services

http://www.emdmillipore.com/techservices

To contact by phone
For North America: Toll-Free US: 1-(800) 221-1975 or 1-(781) 533-8045
Outside North America, contact your local office
http://www.emdmillipore.com/offices
## WELL MAP

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<td>A</td>
<td>0 pg/mL Standard (Background)</td>
<td>Standard 4</td>
<td>QC-2 Control</td>
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<td>B</td>
<td>0 pg/mL Standard (Background)</td>
<td>Standard 4</td>
<td>QC-2 Control</td>
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<td>G</td>
<td>Standard 3</td>
<td>QC-1 Control</td>
<td>Etc.</td>
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<td>Standard 3</td>
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