Total c-Met/HGFR Magnetic Bead MAPmate™

Cat. # 46-650MAG

INTRODUCTION

The MILLIPLEX® MAP Total c-Met/HGFR Magnetic Bead MAPmate™ pair is used in conjunction with the MILLIPLEX® MAP Cell Signaling Buffer and Detection Kit (Catalog # 48-602MAG) to detect the presence of Total c-Met/HGFR in cell lysates using the Luminex® 100™ IS, 200™, or HTS system. Each MAPmate™ pair is ordered individually and may be combined for simultaneous multiplex analysis of cellular events. The MILLIPLEX® MAP Cell Signaling Buffer and Detection Kit is ordered separately and consists of a common set of reagents needed for performing MAPmate™ assays. The detection assay is a rapid, convenient alternative to Western Blotting and immunoprecipitation procedures. Each kit contains sufficient reagents for 100 individual assays. The MILLIPLEX® MAP HeLa Cell Lysate: Unstimulated included in the MILLIPLEX® MAP Cell Signaling Buffer and Detection Kit may be utilized as an unstimulated control for this target.

Important note: For a detailed protocol on Cell Signaling Detection Procedures please see the instruction booklet for the MILLIPLEX® MAP Cell Signaling Buffer and Detection Kit (Catalog # 48-602MAG).

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.

REAGENTS SUPPLIED

REAGENTS SUPPLIED	CATALOG NUMBER	LUMINEX® BEAD #	VOLUME	QUANTITY
MILLIPLEX® MAP Anti-c-Met/HGFR Magnetic Beads (20X)	42-650M	29	180 µL	1 tube
MILLIPLEX® MAP Anti- Total c-Met/HGFR, Biotin (20X)	44-650M	n/a	180 µL	1 tube

STORAGE CONDITIONS UPON RECEIPT

- Recommended storage for kit components is 2-8°C.
- Once the control lysates have been reconstituted, immediately transfer contents into polypropylene vials. **DO NOT STORE RECONSITUTED CONTROLS IN LYOPHILIZATION VIALS**. For long-term storage, freeze reconstituted standards and controls at ≤ -70°C. Aliquot if needed. Avoid freeze/thaw cycles.
- **DO NOT FREEZE Antibody-Immobilized Beads, Detection Antibody, and Streptavidin-Phycoerythrin.**

MATERIALS REQUIRED BUT NOT PROVIDED

Reagents

- MILLIPLEX® MAP Cell Signaling Buffer and Detection Kit (EMD Millipore Catalog # 48-602MAG)
- Protease inhibitors (EMD Millipore Catalog # 535140 or similar product)
- Coomassie or BCA-based total protein assay (EMD Millipore Catalog # 71285 or similar product) or an assay normalization control, such as the GAPDH (EMD Millipore Catalog # 46-667MAG) MAPmate™ or β-Tubulin (EMD Millipore Catalog # 46-713MAG) MAPmate™
- Luminex® Sheath Fluid (20 L, EMD Millipore Catalog # SHEATHFLUID) or Luminex® Drive Fluid (EMD Millipore Catalog # MPXDF-4PK)
- 10X Assay Buffer 1 (EMD Millipore Catalog # MPEQ-AB) if using a magnetic plate washer

Instrumentation / Materials

- Adjustable Pipettes with Tips capable of delivering 25 µL to 1000 µL
- Multichannel Pipettes capable of delivering 25 µL to 200 µL
- Reagent Reservoirs
- Polypropylene Microfuge Tubes
- Rubber Bands
- Aluminum Foil
- Absorbent Pads
- Laboratory Vortex Mixer
- Sonicator (Branson Ultrasonic Cleaner Model # B200 or equivalent)
- Titer Plate Shaker (Lab-Line Instruments Model # 4625 or equivalent)
- Luminex® 200™, HTS, FLEXMAP 3D®, or MAGPIX® with xPONENT® software by Luminex® Corporation
- Plate Stand (EMD Millipore Catalog #MX-STAND, if using filter plate)
- Filter devices for clearing lysates
  - 2 mL or greater, EMD Millipore Catalog # SLHVX13NL
  - 0.5 – 2 mL, EMD Millipore Catalog # UFC40DV25
  - Less than 0.5 mL, EMD Millipore Catalog # UFC30DV25
  - For 96 well plates, EMD Millipore Catalog # MSBVN1210
- If using the filter plate, a Vacuum Filtration Unit (EMD Millipore Vacuum Manifold Catalog # MSVMHTS00 or equivalent with EMD Millipore Vacuum Pump Catalog # WP6111560 or equivalent). Consult Filter Plate Immunoassay Protocol.
- Use of a hand-held Magnetic Separation Block (EMD Millipore Catalog # 40-285 or equivalent) is recommended. If using an Automatic Plate washer for magnetic beads (BioTek® ELx405, EMD Millipore Catalog # 40-015 or equivalent), consult the Plate Washer Use section at the end of the Filter Plate Immunoassay Protocol.

SAFETY PRECAUTIONS

- All tissue 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 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 Assay Buffer provided.
- Any unused mixed Antibody-Immobilized Beads may be stored in the mixing vial at 2-8°C for up to one week**.**
- 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 filter plate using 3 alignment discs. When reading the assay on FLEXMAP 3D®, adjust probe height according to the protocols recommended by Luminex® to the kit filter plate using 1 alignment disc. When reading the assay on MAGPIX®, adjust probe height according to the protocols recommended by Luminex® to the kit filter plate using 2 alignment discs.
- For FLEXMAP 3D® when using the solid plate in the kit, the final suspension should be in 150 µL and 75 µL should be aspirated.

TECHNICAL GUIDELINES (cont.)

- Vortex all reagents well before adding to plate.
- The suggested working range of protein concentration for the assay is 1 to 25 µg of total protein/well (25 µL/well at 40 to 1000 µg/mL). A total protein amount of 20 µg/ well is generally a good starting point for lysates for which target protein expression levels are unknown.

PREPARATION OF LYOPHILIZED MILLIPLEX® MAP CELL LYSATE

MILLIPLEX® MAP Cell Lysates as unstimulated and stimulated controls

1. Reconstitute each of the lyophilized cell lysates in 100 µL of ultrapure water, for each vial. This will yield 100 µL of lysate at a total protein concentration of 2 mg/mL.
2. Gently vortex and incubate the reconstituted lysates for 5 min at RT (store on ice).
3. Pipette 150 µL of MILLIPLEX® MAP Assay Buffer 2 to each cell lysate vial and vortex mix. The cell lysate is now prepared for use in the MILLIPLEX® MAP Magnetic Bead assay.
4. If desired, unused lysate may be stored in its original container at -80°C for up to one month. For long-term storage, freeze reconstituted standards and controls at ≤ -70°C. Aliquot if needed. Avoid freeze/thaw cycles.

SINGLE AND MULTIPLEX ANALYSIS

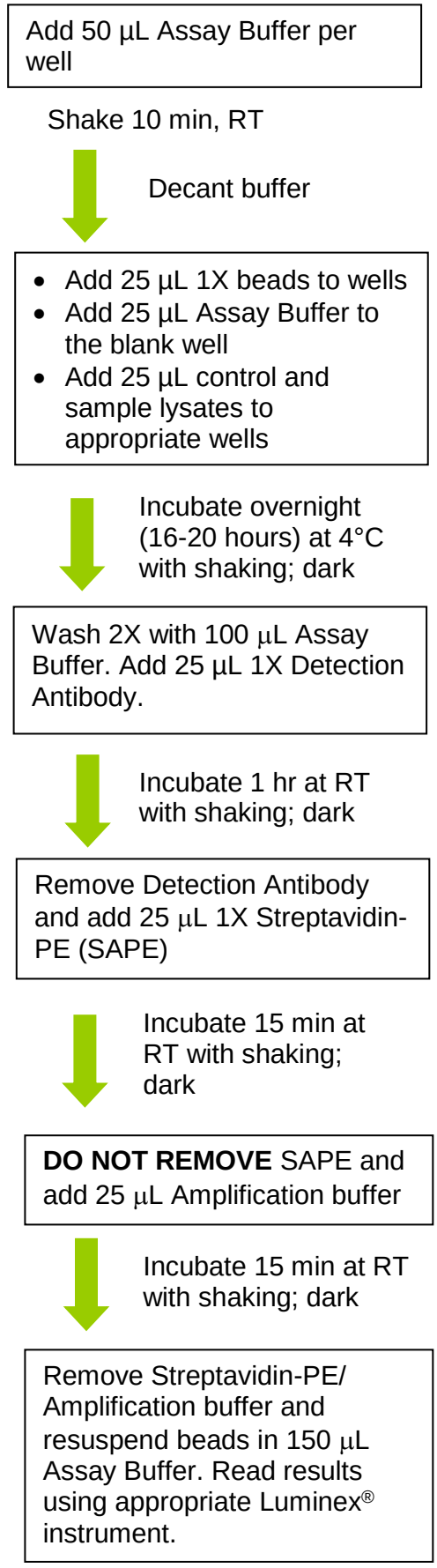
The recommended lysis and assay buffers for single or multi-plex analysis of Total c-Met/HGFR Magnetic Bead MAPmate™ are MILLIPLEX® MAP Lysis Buffer (Catalog # 43-040) and MILLIPLEX® MAP Assay Buffer 2 (Catalog # 43-041). Both buffers are included in the MILLIPLEX® MAP Cell Signaling Buffer and Detection Kit (Catalog # 48-602MAG). For complete cell signaling assay and cell lysis protocols refer to the MILLIPLEX® MAP Cell Signaling Buffer and Detection Kit instructions.

General Guidelines for MAPmate™ multiplexing:

- Phospho-specific and total Magnetic Bead MAPmates™ should not be plexed together, e.g. total GSK3β and phospho-GSK3β (Ser9)
- Phospho STAT3 (Ser727) and Phospho STAT3 (Tyr705) MAPmates™ should not be plexed together
- GAPDH and β-tubulin MAPmates™ can be used for normalization with any of the MAPmates™

IMMUNOASSAY PROTOCOL (96-well Solid Plate and Hand-held Magnetic Separation Block)

1. Dilute filtered lysates at least 1:1 in MILLIPLEX® MAP Assay Buffer 2. The suggested working range of protein concentration for the assay is 1 to 25 µg of total protein/well (25 µL/well at 40 to 1,000 µg/mL).
2. Add 50 µL of Assay Buffer 2 into each well of the plate. Cover and mix on a plate shaker for 10 minutes at room temperature (20-25°C).
3. Decant Assay Buffer and remove the residual amount from all wells by inverting the plate and tapping it smartly onto absorbent towels several times.
4. Vortex the **1X** bead suspension for 10 seconds. Add 25 µL of 1X bead suspension to each well.
5. Add 25 µL of Assay Buffer 2, reconstituted control cell lysates or prepared sample lysates to appropriate wells and incubate overnight (16-20 hours) at 2-8°C on a plate shaker (600-800 rpm) protected from light.
6. Attach handheld magnetic separation block to plate, allow 60 seconds for beads to settle and decant samples and controls.
7. Remove plate from magnetic separation block and wash plate with 100 µL Assay Buffer 2 per well (see **WASHING NOTE** below). Repeat for a total of two washes.
8. Add 25 µL/well of **1X** MILLIPLEX® MAP Detection Antibody.
9. Seal, cover with lid and incubate with agitation on a plate shaker for 1 hour at room temperature (20-25°C).
10. Attach Magnetic Separation Block, wait for 60 seconds and decant Detection Antibody.
11. Add 25 µL of 1X MILLIPLEX® MAP Streptavidin-Phycoerythrin (SAPE).
12. Seal, cover with lid and incubate with agitation on a plate shaker for 15 minutes at room temperature (20-25°C).
13. **DO NOT REMOVE SAPE**. Add 25 µL of MILLIPLEX® MAP Amplification Buffer to each well.
14. Seal, cover with lid and incubate with agitation on a plate shaker for 15 minutes at room temperature (20-25°C).
15. Attach Magnetic Separation Block, wait for 60 seconds and decant SAPE /Amplification buffer.
16. Suspend beads in 150 µL of MILLIPLEX® MAP Assay Buffer 2, and mix on plate shaker for 5 minutes, Analyze using the Luminex® system.



WASHING NOTE: For hand-held magnet, 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 100 µL of Assay Buffer by removing plate from magnet, adding Assay 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.

INSTRUMENT 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: These assays cannot be performed on any instruments running Luminex® IS 2.3 or Luminex® 1.7 software.

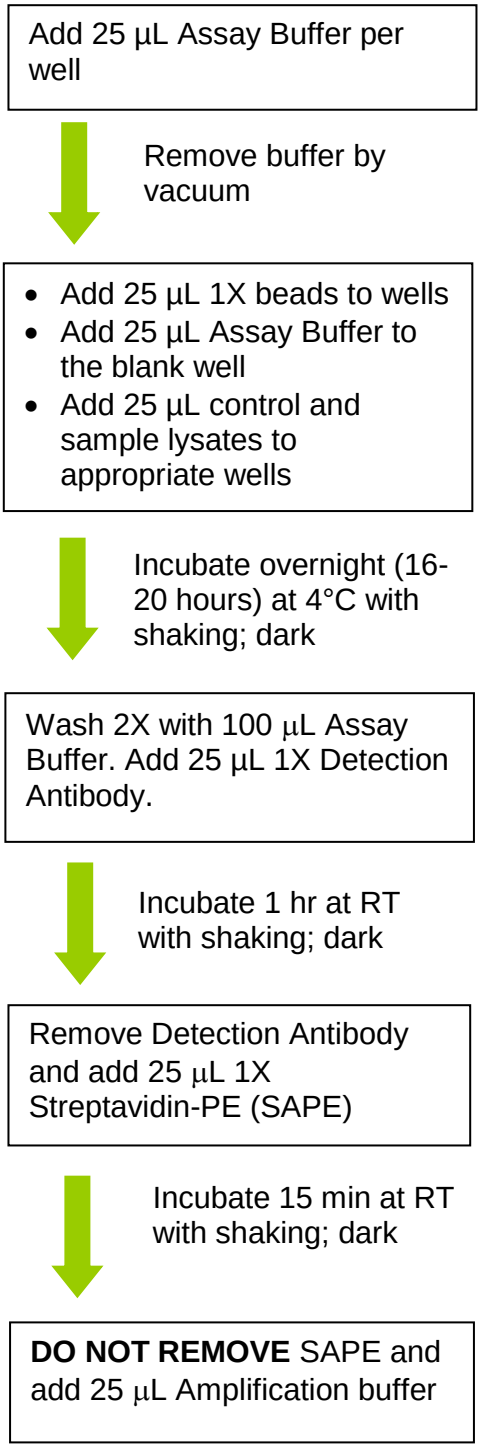
The Luminex® probe height must be adjusted to the plate provided in the kit. Please use EMD Millipore Catalog # MAG-PLATE, if additional plates are required for this purpose.

Events:	50 per bead
Sample Size:	100 µL
Gate Settings:	8,000 to 15,000
Reporter Gain:	Default (Low PMT)
Time Out:	60 seconds
Bead Region:	29

Filter Plate Immunoassay Protocol

NOTE: This protocol requires the use of the included 96-well Filter plate and a Vacuum Manifold (EMD Millipore Vacuum Manifold Catalog # MSVMHTS00 or equivalent with EMD Millipore Vacuum Pump Catalog # WP6111560).

1. Dilute filtered lysates at least 1:1 in MILLIPLEX® MAP Assay Buffer 2. The suggested working range of protein concentration for the assay is 1 to 25 µg of total protein/well (25 µL/well at 40 to 1,000 µg/mL).
2. Pre-wet filter plate with 25 µL/well of MILLIPLEX® MAP Assay Buffer 2. Remove by vacuum filtration by placing the filter plate over a vacuum manifold and gently applying vacuum. Gently blot the bottom of the filter plate on a paper towel to remove excess liquid.
3. Vortex the **1X** bead suspension for 10 seconds. Add 25 µL of 1X bead suspension to each well.
4. Add 25 µL of Assay Buffer 2, reconstituted control cell lysates or sample lysates to appropriate wells and incubate overnight (16-20 hours) at 2-8°C. Seal, cover with lid and incubate with agitation on a plate shaker at 600-800 rpm.
5. Remove the lysate by vacuum filtration.
6. Add 100 µL/well of MILLIPLEX® MAP Assay Buffer 2. Remove buffer by vacuum filtration and gently blot the bottom of the filter plate on a paper towel. Repeat this step again for a total of two washes.
7. Add 25 µL/well of **1X** MILLIPLEX® MAP Detection Antibody.
8. Seal, cover with lid and incubate with agitation on a plate shaker for 1 hour at room temperature (20-25°C).
9. Remove Detection Antibody by vacuum and gently blot the bottom of the filter plate on a paper towel.
10. Add 25 µL of 1X MILLIPLEX® MAP Streptavidin-Phycoerythrin (SAPE).
11. Seal, cover with lid and incubate with agitation on a plate shaker for 15 min at room temperature (20-25°C).
12. **DO NOT REMOVE** SAPE. Add 25 µL of MILLIPLEX® MAP Amplification Buffer to each well.



13. Seal, cover with lid and incubate with agitation on a plate shaker for 15 min at room temperature (20-25°C).
14. Remove MILLIPLEX® MAP SAPE /Amplification buffer by vacuum filtration and gently blot the bottom of the filter plate on a paper towel.
15. Resuspend beads in 150 µL of MILLIPLEX® MAP Assay Buffer 2, and mix on plate shaker for 5 minutes.
16. Analyze using the Luminex® system.

Incubate 15 min at RT with shaking; dark

Remove Streptavidin-PE/ Amplification buffer and resuspend beads in 150 µL Assay Buffer. Read results using appropriate Luminex® instrument.

Plate Washer Use

The use of a plate washer is not a recommended method of washing for cell signaling assays. Deterioration of assay performance and well-to-well variability has been noted when using plate washers. If desired, MPEQ-AB may be purchased and used as a general wash buffer with plate washers. MPEQ-AB should be diluted to 1X for use in plate washers. Follow standard protocol wash instructions when using a plate washer (2 washes after sample incubation). Contact EMD Millipore Tech Service if additional instructions are required.

REPRESENTATIVE DATA

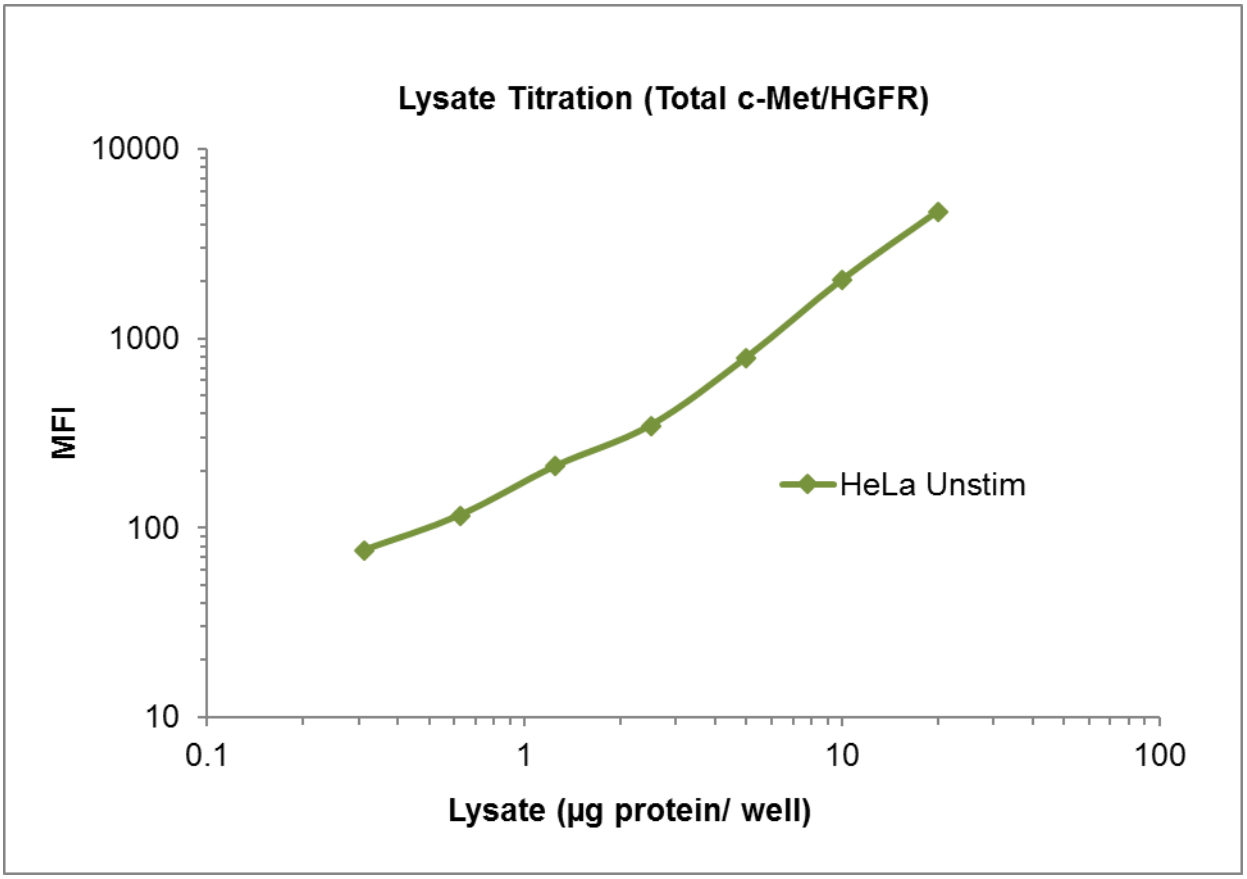


Figure 1. Detection of Total c-Met/HGFR in HeLa cells. HeLa untreated cell lysate was lysed in MILLIPLEX® MAP Lysis Buffer containing protease inhibitors. 1:1 dilutions of cell lysates were diluted in MILLIPLEX® MAP Assay Buffer 2 and assayed according to the assay protocol (lysate incubation at 4ºC overnight). The Median Fluorescence Intensity (MFI) was measured with the Luminex® system. The figures represent the average of triplicate wells.

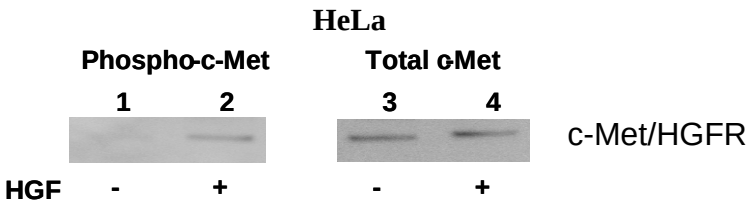


Figure 2. Immunoprecipitation/Western Blot analysis of Total c-Met/HGFR in HeLa cells. HeLa cells were grown to confluence and then lysed in MILLIPLEX® MAP Lysis Buffer with protease inhibitors. 100 µg Non-treated (NT) or HGF-treated HeLa cell lysates were mixed with c-Met/HGFR capture antibody beads to immunoprecipitate c-Met/HGFR from each cell lysate. The immunoprecipitated proteins were separated on SDS-PAGE, transferred to nitrocellulose, and probed with biotin labeled Total c-Met/HGFR detection antibody. The proteins were imaged using Streptavidin-HRP and chemiluminescent substrate.

ORDERING INFORMATION

To place an order or to obtain additional information about our immunoassay products, please contact your Customer Service or Technical Support Specialist.

Contact information for each region can be found on our website:

emdmillipore.com/contact

Conditions of Sale

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

Safety Data Sheets (SDS)

Safety Data Sheets for EMD Millipore products may be downloaded through our website at emdmillipore.com/msds.