Human Oxidative Stress Magnetic Bead Panel

96-Well Plate Assay

Cat. # H0XSTMAG-18K

MILLIPLEX® MAP

HUMAN OXIDATIVE STRESS MAGNETIC BEAD PANEL
96-Well Plate Assay

 # H0XSTMAG-18K

TABLE OF CONTENTS	PAGE

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

INTRODUCTION

Oxidative stress is the result of an imbalance between the production of reactive oxygen species (ROS) and the removal of their reactive intermediates. ROS are products of the Oxidative Phosphorylation pathway located in the mitochondria, and are chemically reactive oxygen molecules that include: hydrogen peroxide, nitric oxide, superoxide, and other hydroxyl radicals. In response to ROS damage, cells defend themselves by clearing the ROS molecules through enzymes such as superoxide dismutases, catalases, glutathione peroxidases and peroxiredoxins. In normal conditions, superoxide dismutase and catalase convert the superoxide and hydrogen peroxide into oxygen and water to minimize the damage to the cells. However, when the ROS clearance pathway is impaired, accumulation of ROS can cause oxidative stress to the cells and induce DNA damage, lipid peroxidation, and enzyme inactivation. Oxidative stress is involved in chronic fatigue syndrome, cardiovascular and neurodegenerative diseases. Brain tissue, in particular, is highly susceptible to oxidative stress due to its characteristics of high levels of oxygen consumption rate, low levels of antioxidants, and low levels of regenerative capacity. On the other hand, ROS can be beneficial, as they are utilized by the immune system as a mechanism to attack and kill pathogens. Some ROS molecules are important for cell signaling as secondary messengers. Therefore, the ability to monitor the oxidative stress level and the balance between ROS production and clearance within cells is a key element to the study of many diseases.

The MILLIPLEX® MAP Human Oxidative Stress Magnetic Bead Panel includes Catalase, PRX2, SOD1, SOD2, and TRX1, and allows the study of these multiple targets simultaneously in one reaction well.

MILLIPLEX® MAP offers the broadest selection of analytes across a wide range of disease states and species. Once the analytes of interest have been identified, you can rely on the quality that we build into each kit to produce results you can trust. In addition to the assay characteristics listed in the protocol, other performance criteria evaluated during the validation process include: cross-reactivity, dilution linearity, kit stability, and sample behavior (e.g. detectability and stability).

This MILLIPLEX® MAP kit includes:

- Stimulated and unstimulated cell lysates provided to qualify assay performance
- Comparison of lysate lots to a reference lot to ensure lot-to-lot consistency
- Detection antibody cocktails designed to yield consistent analyte profiles within panel

In addition each kit meets stringent manufacturing criteria to ensure batch-to-batch reproducibility. The MILLIPLEX® MAP Human Oxidative Stress Magnetic Bead Panel thus enables you to focus on the therapeutic potential of oxidative stress pathways. 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 Oxidative Stress Magnetic Bead Panel is part of the most versatile system available for oxidative stress pathway research. From our single to multiplex biomarker solutions, we partner with you to design, develop, analytically validate and build the most comprehensive library available for protein detection and quantitation.

- MILLIPLEX® MAP offers you:
  - A convenient "all-in-one" box format that gives you the assurance that you will have all the necessary reagents you need to run your assay.

EMD Millipore's MILLIPLEX® MAP Human Oxidative Stress Magnetic Bead Panel is available as a premixed kit and is to be used for the simultaneous detection of Catalase, PRX2, SOD1, SOD2, and TRX1 in human cell lysate or tissue extract.

Full names and alternative names of analytes:

Catalase: Catalase
PRX2: Peroxiredoxin 2, PRDX2
SOD1: Superoxide dismutase 1, Superoxide dismutase [Cu-Zn]
SOD2: Superoxide dismutase 2, MnSOD
TRX1: Thioredoxin

This kit is 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.
- Once the lysate controls have been reconstituted, immediately transfer contents into polypropylene vials. **DO NOT STORE RECONSITUTED CONTROLS IN GLASS VIALS**. For long-term storage, freeze reconstituted controls at ≤ -70°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

Reagents Supplied	Catalog Number	Volume	Quantity
HeLa Cell Lysate: Unstimulated	47-205	lyophilized	1 vial
Set of one 96-Well Plate with 2 sealers	----------	----------	1 plate 2 sealers
Assay Buffer	43-010	55 mL	1 bottle
Cell Lysis Buffer	43-045	12 mL	1 bottle
10X Wash Buffer Note: Contains 0.05% Proclin	L-WB	60 mL	1 bottle
Human Oxidative Stress Panel Pre-mixed Magnetic Beads	H0XSTPMX5-MAG	3.5 mL	1 bottle
Human Oxidative Stress Panel Detection Antibodies	H0XST-1018	5.5 mL	1 bottle
Streptavidin-Phycoerythrin	MC-SAPE6	5.5 mL	1 bottle

Human Oxidative Stress Magnetic Bead Panel Antibody Immobilized Magnetic Beads:

Bead/Analyte Name	Luminex® Magnetic Bead Region
Catalase	27
PRX2	46
SOD1	22
SOD2	39
TRX1	42

MATERIALS REQUIRED BUT NOT PROVIDED

Reagents

1. Protease inhibitors (Catalog # 535140 or similar product)
2. Phosphatase inhibitors (Catalog # 524629 or similar product)
3. Bradford or BCA-based total protein assay (Catalog # 71285 or similar product)
4. Luminex® Sheath Fluid (EMD Millipore Catalog # SHEATHFLUID) or Luminex® Drive Fluid (EMD Millipore Catalog # MPXDF-4PK)

MATERIALS REQUIRED BUT NOT PROVIDED (continued)

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.

Note: See Full Labels of Hazardous components on next page.

Full labels of hazardous components in this kit:

Ingredient, Cat #		Full Label	
HeLa Cell Lysate	47-205		**Danger.** Causes serious eye damage. Harmful to aquatic life with long lasting effects. Avoid release to the environment. Wear eye protection. IF IN EYES: Rinse cautiously with water for several minutes. Remove contact lenses, if present and easy to do. Continue rinsing. Get medical advice/ attention.
Wash Buffer	L-WB		**Warning.** May cause an allergic skin reaction. Wear protective gloves. IF ON SKIN: Wash with plenty of soap and water.

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 equilibrate to room temperature (20-25°C) before use in the assay.
- Incomplete washing can adversely affect the assay outcome. All washing must be performed using the provided Wash Buffer.
- The reconstituted lysate control must be used within 1 hour of preparation. Discard any unused lysate control except the stock which may be stored at ≤ -70°C for up to 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 Premixed Antibody-Immobilized Beads may be stored at 2-8°C for up to one month.
- The plate should be read immediately after the assay is finished. If the plate cannot be read immediately, seal the plate and 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.
- Vortex all reagents well before adding to plate.

SAMPLE COLLECTION AND STORAGE

A. Considerations for Cell Stimulation

1. Depending on the experimental design, cells can be treated with growth factors (e.g. insulin), cytokines (e.g. TNFα), or other compounds before harvesting the cell lysates. The duration of stimulation in addition to the concentration of the respective factor/compound should be considered since they influence the degree of phosphorylation and protein expression of any given analyte.
2. Cellular responses to growth factors are typically improved when cells have been serum starved prior to treatment.
3. Cell lines will differ in the robustness of their signaling response for any given stimulation.

B. Preparation of Cell Lysates

Cell Lysis Buffer is supplied as **1X** working solution. The Lysis Buffer does ***NOT*** contain protease inhibitors or phosphatase inhibitors. It is recommended that protease inhibitors (Catalog # 535140, available separately) and phosphatase inhibitors (Catalog # 524629, available separately) be added immediately before use.

Suggested cell lysis protocol for adherent cells

1. After treatments, rinse cells with ice cold Phosphate Buffered Saline (PBS) and drain off PBS.
2. Add ice cold Cell Lysis Buffer with freshly added phosphatase and protease inhibitors to cells (0.6 mL per 150 mm dish, 0.3 mL per 100 mm dish, or 0.1 mL per well of 24-well plate).
3. Scrape adherent cells off the dish with a cell scraper. Transfer the cell suspension into a centrifuge tube and gently rock for 15-30 minutes at 4°C.
4. Centrifuge the lysate at 14,000 x g for 20 minutes at 4–8°C. Immediately transfer the cleared cell lysate (supernatant) into fresh pre-chilled microcentrifuge tubes.
5. Aliquot and store the lysate at ≤ -70°C. The lysate should be stable for several months.
6. It is recommended that the lysate be diluted at least 1:10 in PBS for determining the protein concentration with Coomassie Bradford assays or 1:4 for BCA assays.

Suggested cell lysis protocol for non-adherent cells

1. Pellet the cells by centrifugation (500 – 1000 x g) in a tabletop centrifuge for 5 minutes.
2. Wash the cells in ice cold PBS.
3. Add ice cold Cell Lysis Buffer with freshly added phosphatase and protease inhibitors to cells (1 mL per 1 x 10^7 cells).
4. Gently rock the lysate for 15-30 minutes at 4°C.
5. Centrifuge the lysate at 14,000 x g for 20 minutes at 4–8°C. Immediately transfer the cleared cell lysate (supernatant) into fresh pre-chilled microcentrifuge tubes.
6. Aliquot and store the lysate at ≤ -70°C. The lysate should be stable for several months.
7. It is recommended that the lysate be diluted at least 1:10 in PBS for determining the protein concentration with Coomassie Bradford assays or 1:4 for BCA assays.

Cell lysis protocol for cells in sterile 96-well tissue culture plates

Adherent or non-adherent cells seeded or grown in sterile 96-well tissue culture grade plates (see supplemental protocols) can be washed, treated, and lysed in the same plate, but need to be filtered in a separate 96-well filter plate. Wash the cells by centrifugation in a micro plate carrier for 2 minutes at 500 x g.

Remove the supernatant via aspiration and add 100 µL of ice-cold PBS.

1. Centrifuge and remove supernatant via aspiration.
2. Add 30-50 µL/well of ice cold Cell Lysis Buffer with freshly added phosphatase and protease inhibitors.
3. Place the plate on an orbital shaker (600 – 800 rpm) for 10-15 minutes at 4°C.
4. Transfer the lysate to a 96-well filter plate that has been pre-wetted with Cell Lysis Buffer.
5. Place a low protein binding, 96-well round bottom or V-bottom plate underneath the filter plate.
6. Centrifuge the plates in a micro plate carrier for 5 minutes at 500 x g.
7. Store the filtered lysate at ≤ -70°C until ready for use.
8. It is recommended that the lysate be diluted at least 1:10 in PBS for determining the protein concentration with Coomassie Bradford assays or 1:4 for BCA assays.

C. Preparation of tissue extracts

Cell Lysis Buffer is supplied as **1X** working solution. The Lysis Buffer does ***NOT*** contain protease inhibitors or phosphatase inhibitors. It is recommended that protease inhibitors (Catalog # 535140, available separately) and phosphatase inhibitors (Catalog # 524629, available separately) be added immediately before use.

Suggested protocol for tissue extracts preparation

1. Weigh out the appropriate amount of tissue. Wash the sample tissue twice with ice-cold Phosphate Buffered Saline (PBS).
2. Mince the tissue with a scalpel and place in pre-chilled 2 mL Dounce homogenizer. Add **up to** 2.0 mL Cell Lysis Buffer with freshly added phosphatase and protease inhibitors to the tissue in the homogenizer.
3. To rupture the cells, perform 20–50 Dounce strokes, use pestle A (large clearance) for the initial strokes, then use pestle B (small clearance) for the remaining strokes. Tissue homogenate can be stored at ≤-70°C in aliquots at this point.
4. To prepare tissue extracts, estimate the volume of the tissue homogenate and add 4 extra volumes of ice-cold Cell Lysis Buffer with freshly added phosphatase and protease inhibitors.
5. Incubate on ice for 30 minutes with occasional vortexing. Centrifuge the tissue extracts at 14,000 x g for 20 minutes at 4–8°C. Immediately aliquot the cleared supernatant into fresh microcentrifuge tubes and discard the pellet.
6. Store the tissue extract aliquots at ≤ -70°C. The tissue extract should be stable for several months.
7. It is recommended that the tissue extract be diluted at least 1:10 with PBS for determining the protein concentration with Coomassie Bradford assays or 1:4 for BCA assays.

PREPARATION OF REAGENTS FOR IMMUNOASSAY

A. Preparation of Antibody-Immobilized Beads

Sonicate the Human Oxidative Stress Panel Pre-mixed Magnetic Beads (1X) for 30 seconds and vortex for one minute just prior to use. The 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.)

B. Preparation of Wash Buffer

Bring the 10X Wash Buffer to room temperature and mix to bring all salts into solution. Dilute 60 mL of 10X Wash Buffer with 540 mL deionized water. Store the unused portion at 2-8°C for up to one month.

C. Preparation of lyophilized HeLa Cell Lysate: Unstimulated (Catalog # 47-205)

HeLa Cell Lysate: Unstimulated (# 47-205) is provided as a lyophilized stock of cell lysate prepared from unstimulated HeLa cells and is used as a positive control.

1. Reconstitute the lyophilized cell lysate in 100 µL of Ultrapure water; this will yield 100 µL of lysate at a total protein concentration of 2 mg/mL. If desired, unused lysate may be stored in polypropylene vials at ≤ -70°C for up to one month.
2. Gently vortex and incubate the reconstituted lysate for 5 min at RT (store on ice).
3. Mix 20 µL of the 2 mg/mL cell lysate with 80 µL of Assay Buffer in a microcentrifuge tube. The prepared cell lysate is now ready for use.

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 equilibrate to room temperature (20-25°C) before use in the assay.
- Diagram the placement of Blanks, Lysate Control, 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 to ensure that bottom of the plate does not touch any surface.

1. Dilute pre-cleared lysates **at least 1:1** in Assay Buffer. The suggested working range of protein concentration for the assay is **5 to 20** µg of total protein/well (25 µL/well at 200 to 800 µg/mL). **No additional dilution is necessary for the prepared Lysate Controls.**
2. 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).
3. Decant Assay Buffer and remove the residual amount from all wells by inverting the plate and tapping it onto absorbent towels several times.
4. Add 25 µL of Assay Buffer to the background wells.
5. Add 25 µL of the Lysate Control into the appropriate wells.
6. Add 25 µL of diluted lysate sample into the appropriate wells.
7. Vortex bead bottle and add 25 µL of the Premixed Beads to each well. (Note: During addition of Beads, shake bead bottle intermittently to avoid settling.)
8. Seal the plate with a plate sealer, cover it with the lid. Wrap the plate with foil and incubate with agitation on a plate shaker 2 hours at room temperature (20-25°C).

Add 200 µL Assay Buffer per well

↓ Shake 10 min, RT

↓ Decant

- Add 25 µL Assay Buffer to background wells
- Add 25 µL Lysate Control to appropriate wells
- Add 25 µL diluted lysate samples to appropriate wells
- Add 25 µL Beads to each well

↓ Incubate 2 hr at RT

9. Gently remove well contents and wash plate 3 times following instructions listed in the **PLATE WASHING** section.

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

11. Seal, cover with lid and incubate with agitation on a plate shaker for 1 hour at room temperature (20-25°C).

12. Gently remove well contents and wash plate 3 times following instructions listed in the **PLATE WASHING** section.

13. Add 50 µL Streptavidin-Phycoerythrin to each well.

14. Seal, cover with lid 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 3 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 on a plate shaker for 5 minutes.

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

18. Save and analyze the Median Fluorescence Intensity (MFI) data. (Note: make sure all lysate samples in comparison are diluted by the same factor.)

Remove well contents and wash 3X with 200 µL Wash Buffer

↓

Add 50 µL Detection Antibodies per well

↓ Incubate 1 hr at RT

↓ Remove well contents and wash 3X with 200 µL Wash Buffer

Add 50 µL Streptavidin-Phycoerythrin per well

↓ Incubate 30 minutes at RT

↓ Remove well contents and wash 3X with 200 µL Wash Buffer

Add **100** µL Sheath Fluid or Drive Fluid per well

Read on Luminex®:
50 µL, 50 beads per bead set

PLATE WASHING

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.

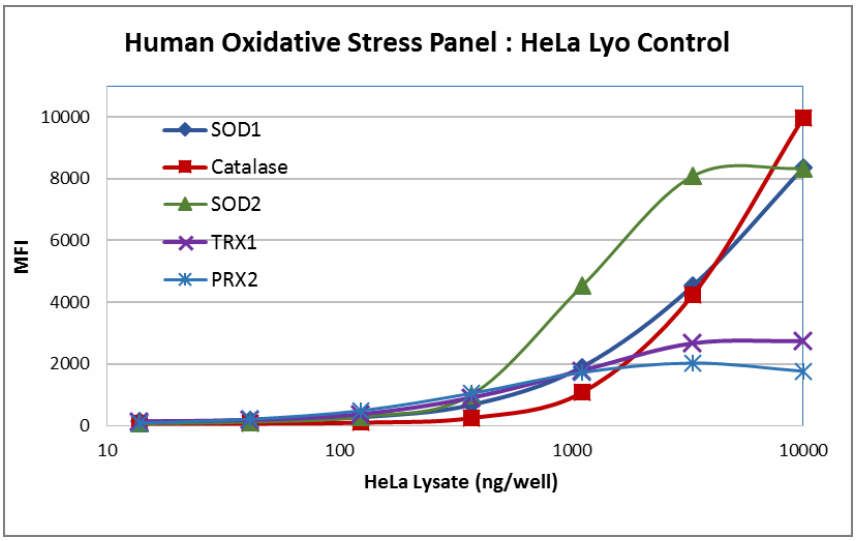
NOTE: These assays cannot be run on any instruments using Luminex® IS 2.3 or Luminex® 1.7 software.

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.

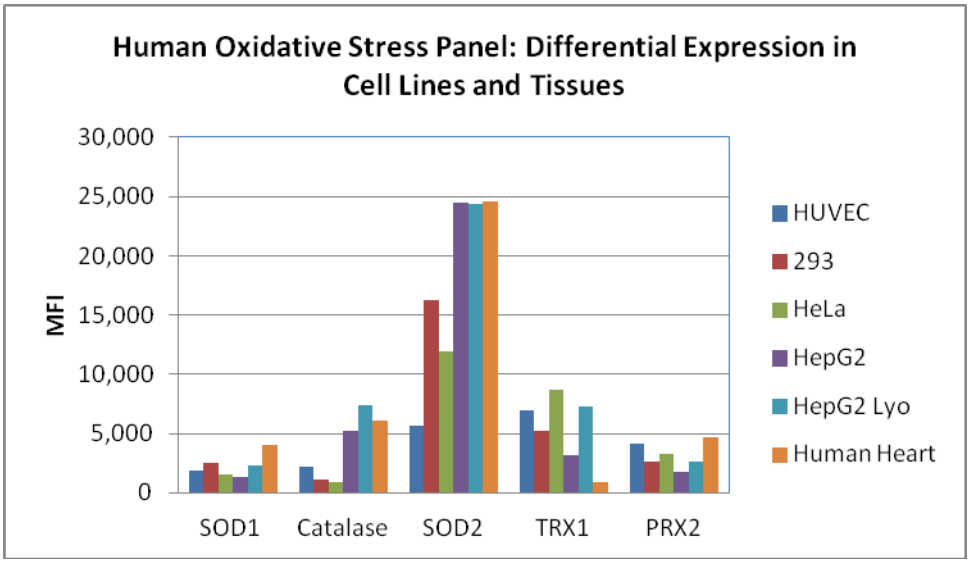
Events:	50 per bead	
Sample Size:	50 µL	
Gate Settings:	8,000 to 15,000	
Reporter Gain:	Default (low PMT)	
Time Out:	60 seconds	
Bead Set:	5-Plex Beads	
	Catalase	27
	PRX2	46
	SOD1	22
	SOD2	39
	TRX1	42

ASSAY CHARACTERISTICS

Representative Data



Human Oxidative Stress Magnetic Bead Panel serial dilution curve. The HeLa control (# 47-205) was prepared according to the procedures described in the protocol and serially diluted (3-fold) with Assay Buffer, then analyzed with the Human Oxidative Stress Magnetic Bead Panel according to the assay protocol. The Median Fluorescence Intensity (MFI) was measured with the Luminex®® system.



Multiplexed analysis of different human cell lines and heart tissue extract with the Human Oxidative Stress Magnetic Bead Panel. Lysates from different human cell lines (HUVEC, HEK 293, HeLa, HepG2) and heart tissue were prepared according to the procedures described in the protocol. 1000 ng of cell line lysates and heart tissue extract were analyzed with the Human Oxidative Stress Magnetic Bead Panel according to the assay protocol. The Median Fluorescence Intensity (MFI) was measured with the Luminex® system.

ASSAY CHARACTERISTICS (continued)

Specificity/Cross-Reactivity

There was no or negligible cross-reactivity between the antibodies for an analyte and any of the other analytes within this panel.

Precision

Intra-assay precision is generated from the mean of the %CVs from 8 reportable results across two different concentrations of analytes in a single assay. Inter-assay precision is generated from the mean of the %CVs from 48 reportable results across two different concentrations of analytes from 6 different assays.

Analyte	Intra-Assay CV	Inter-Assay CV
Catalase	< 10.0%	< 15.0%
PRX2	< 10.0%	< 15.0%
SOD1	< 10.0%	< 15.0%
SOD2	< 10.0%	< 15.0%
TRX1	< 10.0%	< 15.0%

TROUBLESHOOTING GUIDE

Problem	Probable Cause	Solution
Insufficient bead count	Plate washer aspiration height set too low	Adjust aspiration height according to manufacturers' instructions.
	Bead mix prepared inappropriately	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.
	Samples cause interference due to particulate matter or viscosity	See above. Also sample probe may need to be cleaned with Alcohol flush, Back flush and washes; or if needed probe should be removed and sonicated.
	Probe height not adjusted correctly	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.
Background is too high	Background wells were contaminated	Avoid cross-well contamination by using sealer appropriately and pipetting with multichannel pipettes without touching reagent in plate.
	Insufficient washes	Increase number of washes.
Beads not in region or gate	Luminex® instrument not calibrated correctly or recently	Calibrate Luminex® instrument based on manufacturer's instructions, at least once a week or if temperature has changed by >3°C.
	Gate settings not adjusted correctly	Some Luminex® instruments (e.g. Bio-Plex®) require different gate settings than those described in the kit protocol. Use instrument default settings.
	Wrong bead regions in protocol template	Check kit protocol for correct bead regions or analyte selection.
	Incorrect sample type used	Samples containing organic solvents or if highly viscous should be diluted or dialyzed as required.
	Instrument not washed or primed	Prime the Luminex® instrument 4 times to remove air bubbles, wash 4 times with sheath fluid or water if there is any residual alcohol or sanitizing liquid.
	Beads were exposed to light	Keep plate and bead mix covered with dark lid or aluminum foil during all incubation steps.

Problem	Probable Cause	Solution
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.
Low signal for positive Lysate Control	Incubations done at inappropriate temperatures, timings or agitation	Assay conditions need to be checked. Add Streptavidin-Phycoerythrin according to protocol.
Sample signals too high and saturated	Calibration target value set too high Plate incubation was too long with Lysate Control and samples Samples contain analyte concentrations higher than the assay dynamic range	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. Samples may require dilution and re-analysis for just that particular analyte.
Sample signals too low	Samples contain no or below detectable levels of analyte	If below detectable levels, it may be possible to use higher sample volume. Check with technical support for appropriate protocol modifications.
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 a vertical 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		
Problem	**Probable Cause**	**Solution**
Filter plate will not vacuum	Vacuum pressure is insufficient	Increase vacuum pressure such that 0.2 mL buffer can be suctioned in 3-5 seconds.
	Samples have insoluble particles	Centrifuge samples just prior to assay setup and use supernatant.
	High lipid concentration	After centrifugation, remove lipid layer and use supernatant.
Plate leaked	Vacuum pressure too high	Adjust vacuum pressure such that 0.2 mL buffer can be suctioned in 3-5 seconds. May need to transfer contents to a new (blocked) plate and continue.
	Plate set directly on table or absorbent towels during incubations or reagent additions	Set plate on plate holder or raised edge so bottom of filter is not touching any surface.
	Insufficient blotting of filter plate bottom causing wicking	Blot the bottom of the filter plate well with absorbent towels after each wash step.
	Pipette touching plate filter during additions	Pipette to the side of plate.
	Probe height not adjusted correctly	Adjust probe to 3 alignment discs in well H6.
	Sample too viscous	May need to dilute sample.

REPLACEMENT REAGENTS	**Catalog #**
HeLa Cell Lysate	47-205
Human Oxidative Stress Panel Pre-mixed Magnetic Beads	H0XSTPMX5-MAG
Human Oxidative Stress Panel Detection Antibodies	H0XST-1018
Assay Buffer	43-010
Cell Lysis Buffer	43-045
Streptavidin-Phycoerythrin	MC-SAPE6
Set of two 96-Well plates with Sealers	MAG-PLATE
10X Wash Buffer	L-WB

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.

WELL MAP

	1	2	3	4	5	6	7	8	9	10	11	12
A	Background	Sample 3										
B	Background	Sample 3										
C	Lysate Control	Etc.										
D	Lysate Control											
E	Sample 1											
F	Sample 1											
G	Sample 2											
H	Sample 2											