



Certificate of Analysis

MILLIPLEX™ MAP Phospho-Histone H2A.X (Ser139) Beadmate™

(100 Assay Points)

Catalog # 46-692

Lot #

Components

MILLIPLEX MAP Anti-Histone H2A.X Beads, Catalog # 42-692, Lot #. One vial containing **131 µL** of anti-Histone H2A.X rabbit IgG conjugated to Luminex® Bead #43 at **2400 beads/µL (20X)** in a proprietary formulation of Tris buffered salts and animal protein containing 0.05% sodium azide as a preservative.

MILLIPLEX MAP Anti-Phospho-Histone H2A.X, Biotin, Catalog # 44-692, Lot #. One vial containing **131 µL** of biotin conjugated anti-Phospho-Histone H2A.X (Ser139) mouse IgG (**20X**) in a proprietary formulation of Tris buffered salts and animal protein containing 0.05% sodium azide as a preservative.

Specificity: The assay recognizes phosphorylated Histone H2A.X (Ser139).

Applications: Optimal antibody pair for detection of Phospho-Histone H2A.X. To be used in conjunction with the **MILLIPLEX MAP Cell Signaling Buffer Kits** (Catalog #s 48-600 or 48-601).

Storage: Store in the **dark** at 4 °C.

FOR RESEARCH USE ONLY

**NOT RECOMMENDED OR INTENDED FOR DIAGNOSIS OF DISEASE IN HUMANS OR ANIMALS
DO NOT USE IN HUMANS OR IN ANIMALS**

Phospho-Histone H2A.X (Ser139) Beadmate™ Description

Use: The Phospho-Histone H2A.X (Ser139) Beadmate™ pair is used in conjunction with **MILLIPLEX MAP Cell Signaling Buffer Kits** (Catalog #s 48-600 or 48-601) to detect the presence of phosphorylated Histone H2A.X (Ser139) in cell lysates using the Luminex® instrument. Each Beadmate™ pair is ordered individually and can be combined for simultaneous multiplex analysis of cellular events. **MILLIPLEX MAP Cell Signaling Buffer Kits** are ordered separately and consist of a common set of reagents needed for using Beadmates™. The detection assay is a rapid, convenient alternative to Western Blotting and immunoprecipitation procedures. Each kit contains sufficient reagents for 100 individual assays.

Important note: For a detailed protocol on Cell Signaling Detection Procedures please see the COA (select the highest lot number) for the **MILLIPLEX MAP Cell Signaling Buffer Kit** available at:

<http://www.millipore.com/catalogue/item/48-600#coa>

Other components required but not included as part of kit are:

- Cell lysates or cell extracts harboring protein(s) of interest
- Vortex mixer
- Plate shaker
- Timer
- Variable volume (5 - 200 μ L) pipette + tips
- Sonication Bath (Catalog # 40-002)
- Millipore multiscreen vacuum manifold (Catalog # MAVM0960R)
- Luminex[®] Instrument
- **MILLIPLEX MAP** Cell Signaling Buffer Kit (Catalog # 48-600) or Cell Signaling Universal Buffer Kit (Catalog # 48-601)

Detection Protocol Summary

The assay procedure is a simple fluorescent bead-based sandwich immunoassay that is sensitive and easy to perform. A cell lysate or other sample is incubated with beads coupled to a Histone H2A.X specific capture antibody overnight at 4°C. The beads are washed and mixed with a biotinylated phospho-Histone H2A.X (Ser139) specific reporter, followed by streptavidin-phycoerythrin. The amount of phosphorylated Histone H2A.X is then quantified using the Luminex[®] Instrument. A sample with unstimulated cell lysate and containing all other components will give the value for any basal levels of phosphorylated Histone H2A.X.

Pre-wet filter plate and add 25 μ L of diluted cell lysate to each well with 25 μ L of 1X Histone H2A.X bead solution.



*overnight; dark
(4°C, shaking)*

Wash with 100 μ L **MILLIPLEX MAP Cell Signaling Assay Buffer 1** and add 25 μ L of 1X Phospho-Histone H2A.X (Ser139) reporter solution.



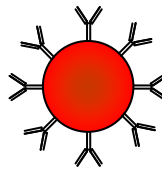
*1 hour; dark
(RT, shaking)*

Remove reporter and add 25 μ L diluted **MILLIPLEX MAP** Streptavidin-Phycoerythrin.

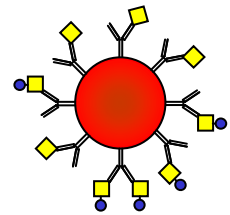


*30 min; dark
(RT, shaking)*

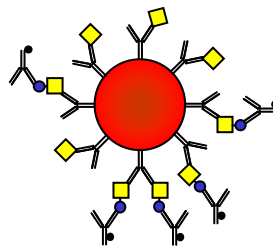
Remove Streptavidin-Phycoerythrin and resuspend in 100 μ L **MILLIPLEX MAP Cell Signaling Assay Buffer 1** and read results on Luminex[®] Instrument



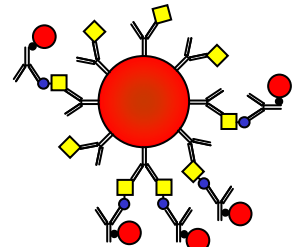
1. Bead with capture antibody



2. Capture antibody binds target proteins



3. Biotinylated reporter binds phosphorylated proteins



4. Streptavidin-PE binds biotinylated reporter antibody and emits fluorescent signal

Preparations for Assay Protocol

Single-plex analysis

The recommended lysis and assay buffers for a single-plex analysis of Phospho-Histone H2A.X (Ser139) Beadmates™ are **MILLIPLEX MAP Cell Signaling Universal Lysis Buffer** (Catalog # 43-040) and **MILLIPLEX MAP Cell Signaling Assay Buffer 1** (Catalog # 43-010). For the cell signaling assay and cell lysis protocols refer to the **MILLIPLEX MAP Cell Signaling Buffer Kit COA** (select the highest lot number) at:

<http://www.millipore.com/catalogue/item/48-600#coa>

Multiplex analysis

The recommended lysis and assay buffers multiplexing Phospho-Histone H2A.X (Ser139) Beadmate™ with other Beadmates™ are **MILLIPLEX MAP Cell Signaling Universal Lysis Buffer** (Catalog # 43-040) and **Cell Signaling Assay Buffer 1** (Catalog # 43-040). Both buffers are included in the **MILLIPLEX MAP Cell Signaling Universal Buffer Kit** (Catalog # 48-601). For the cell signaling assay and cell lysis protocols refer to the **MILLIPLEX MAP Cell Signaling Universal Buffer Kit COA** (select the highest lot number) at: <http://www.millipore.com/catalogue/item/48-601>

For multiplexing Phospho-Histone H2A.X (Ser139) with other Beadmates™ that are *not* compatible with **MILLIPLEX MAP Cell Signaling Assay Buffer 1**, select the optimal lysis and assay buffers using the Buffer Selection Table in the **MILLIPLEX MAP Cell Signaling Buffer Kit COA** (Catalog # 48-600). The cell signaling assay and cell lysis protocols are also provided in the **MILLIPLEX MAP Cell Signaling Buffer Kit COA** at: <http://www.millipore.com/catalogue/item/48-600#coa> (select the highest lot number).

Note: Phospho and Total Beadmates should not be multiplexed together.

Control Cell Lysate Preparation

The suggested working range of protein concentration for the assay is 10 µg of total protein/well (25 µL/well at 0.4 µg/µL).

Sample Cell Lysate Preparation

The suggested working range of protein concentration for the assay is 1 to 25 µg of total protein/well (25 µL/well at 0.04 to 1 µg/µL), depending on the cell type used.

Representative Data:

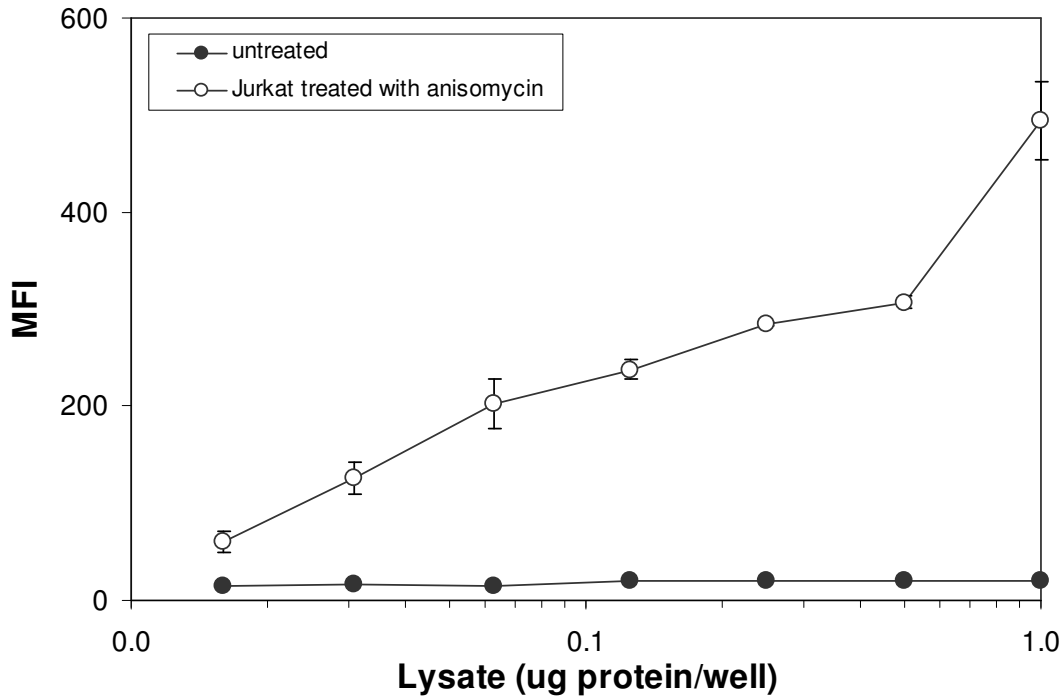


Figure 1. MILLIPEX MAP detection of phospho-Histone H2A.X (Ser139) in Jurkat cell lysate. Jurkat cells were grown to 1×10^6 cells/mL and stimulated with or without 25 μ M anisomycin for 4 hours. Increasing amount of cell lysates (lysed in MILLIPEX MAP Universal Lysis Buffer with protease inhibitors) were incubated overnight at 4°C with anti-H2A.X capture beads. The beads were washed and mixed at room temperature with biotin labeled anti-phospho-Histone H2A.X (Ser139), followed by streptavidin-PE. The Median Fluorescent Intensity (MFI) in triplicate wells was measured using the Luminex® Instrument. This graph shows changes in phosphorylation of Histone H2A.X as detected with Phospho-Histone H2A.X (Ser139) Beadmates™.

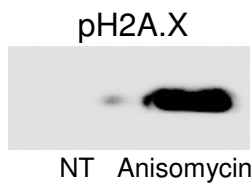


Figure 2. Immunoprecipitation/Western Blot analysis of phospho-Histone H2A.X in Jurkat cells. 20 μ g of Jurkat cell lysates (described in Figure 1) were mixed with capture antibody beads to immunoprecipitate phospho-Histone H2A.X protein from non-treated (NT) and anisomycin-treated cell lysates. The immunoprecipitated proteins were separated on SDS-PAGE, transferred to nitrocellulose, and probed with biotin labeled phospho-Histone H2A.X reporter antibody. The proteins were imaged using Streptavidin-HRP and chemiluminescence.

End-User License Agreement

By purchasing this product, which contains fluorescently labeled microsphere beads authorized by Luminex Corporation, you, the customer, acquire the right under Luminex Corporation'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 Luminex® 100™. One or more of the following US patents covers this product and the use thereof: #6,046,807, #5,981,180.