
Certificate of Analysis

Beadlyte[®] Phospho-JNK/SAPK1 (Thr183/Tyr185) Beadmates[™]
(100 Assay Points)
Catalog # 46-613
Lot # 0608038493

Components

Beadlyte[®] Anti-JNK/SAPK1 Beads, Catalog # 42-613, Lot # 0608038493. One vial containing **125 μ l** of anti-pan JNK/SAPK1 IgG conjugated to Luminex[™] Bead # 16 at **2,000 beads/ μ l (20X)** in a proprietary formulation of Tris-buffered salts and animal protein containing 0.05% sodium azide as a preservative.

Beadlyte[®] Anti-phospho-JNK/SAPK1 (Thr183/Tyr185), Biotin, Catalog # 44-613, Lot # 0608038493. One vial containing **125 μ l** of anti-phospho-JNK/SAPK1 IgG (**20X**) in a proprietary formulation of Tris-buffered salts and animal protein containing 0.05% sodium azide as a preservative.

Specificity: Recognizes human and mouse JNK/SAPK1 phosphorylated on (Thr183/Tyr185).

Applications: Optimal antibody pair for detection of JNK/SAPK1 phosphorylated on Thr183/Tyr185. To be used in conjunction with Beadlyte[®] Cell Signaling Buffer Kits (Catalog #s 48-600 and 48-601).

Storage and Stability: Stable for 1 year at 4°C from date of shipment. Store in the **dark**.

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-JNK/SAPK1 (Thr183/Tyr185) Beadmate[™] Description

Use: The Beadlyte[®] Phospho-JNK/SAPK1 (Thr183/Tyr185) Beadmate[™] pair is used in conjunction with Beadlyte[®] Cell Signaling Buffer Kits (Catalog #s 48-600 or 48-601) to detect the presence of phosphorylated JNK/SAPK1 (Thr183/Tyr185) in cell lysates using the Luminex xMAP[™] system. Each Beadmate[™] pair is ordered individually and can be combined for simultaneous multiplex analysis of cellular events. Beadlyte[®] 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 Beadlyte[®] Cell Signaling Buffer Kit available at:

<http://www.upstate.com/browse/productdetail.asp?ProductId=48-600>

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 xMAP™ System
- Beadlyte® 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 JNK/SAPK1 specific capture antibody overnight. The beads are washed and mixed with a biotinylated phospho-JNK/SAPK1 specific reporter, followed by streptavidin-phycoerythrin. The amount of phospho-JNK/SAPK1 (Thr183/Tyr185) is then quantified using the Luminex xMAP™ System. A sample with unstimulated cell lysate and containing all other components will give the value for any basal phosphorylation of JNK/SAPK1.

Pre-wet filter plate and add 25 μ l of diluted cell lysate to each well with 25 μ l of 1X JNK/SAPK1 bead solution.



*Overnight; dark
(4 °C, shaking)*

Wash with 100 μ l Beadlyte® Cell Signaling Assay Buffer and add 25 μ l of 1X Phospho-JNK/SAPK1 reporter solution.



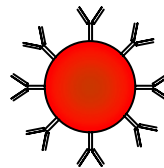
*1 hour; dark
(RT, shaking)*

Remove reporter and add 25 μ l diluted Beadlyte® Streptavidin-Phycoerythrin.

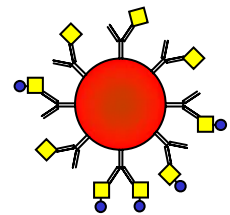


*30 min; dark
(RT, shaking)*

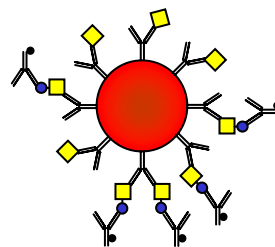
Remove Streptavidin-Phycoerythrin and resuspend in 100 μ l **Beadlyte® Cell Signaling Assay Buffer 1** and read results on Luminex 100.



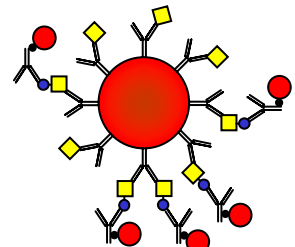
1. Bead with capture antibody



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-JNK/SAPK1 (Thr183/Tyr185) Beadmates™ are Beadlyte® Cell Signaling **Lysis Buffer B** (Catalog # 43-019) and Beadlyte® Cell Signaling **Assay Buffer 2** (Catalog # 43-011). Both buffers are included in the Beadlyte® Cell Signaling Buffer Kit (Catalog # 48-600). For the cell signaling assay and cell lysis protocols refer to the Beadlyte® Cell Signaling Buffer Kit COA (select the highest lot number) at: <http://www.upstate.com/browse/productdetail.asp?ProductId=48-600>.

Multiplex analysis

The recommended lysis and assay buffers multiplexing Phospho-JNK/SAPK1 (Thr183/Tyr185) Beadmates™ with other Beadmates™ are Beadlyte® Cell Signaling Universal Lysis Buffer (Catalog # 43-040) and Cell Signaling Universal Assay Buffer (Catalog # 43-041). Both buffers are included in the Beadlyte® Cell Signaling Universal Buffer Kit (Catalog # 48-601). For the cell signaling assay and cell lysis protocols refer to the Beadlyte® Cell Signaling Universal Buffer Kit COA (select the highest lot number) at: <http://www.upstate.com/browse/productdetail.asp?ProductId=48-601>.

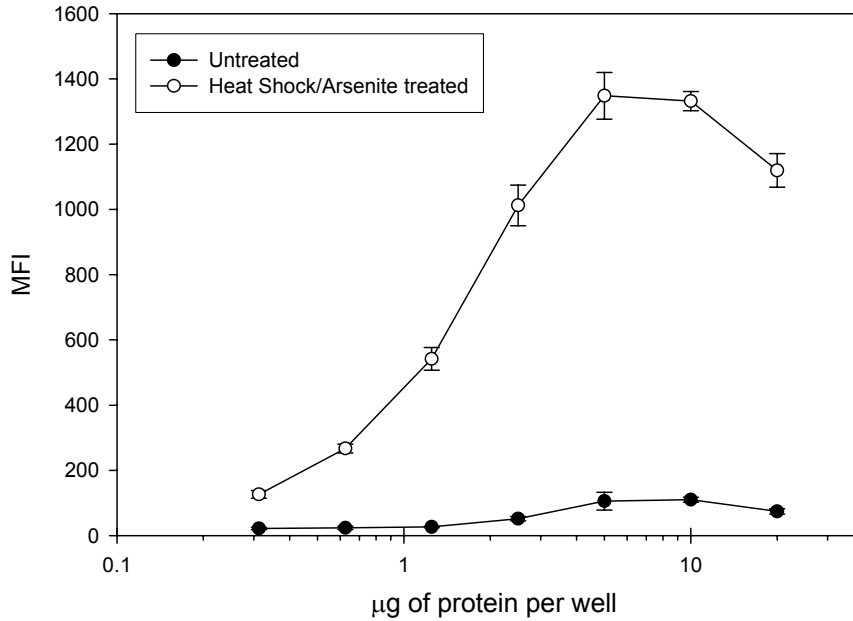
For multiplexing Phospho-JNK/SAPK1 (Thr183/Tyr185) with other Beadmates™ that are *not* compatible with the Universal Buffer System, select the optimal lysis and assay buffers using the Buffer Selection Table in the Beadlyte® Cell Signaling Buffer Kit COA (Catalog # 48-600). The cell signaling assay and cell lysis protocols are also provided in the Beadlyte® Cell Signaling Buffer Kit COA at: <http://www.upstate.com/browse/productdetail.asp?ProductId=48-600> (select the highest lot number).

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

Representative Data:

A.

Detection of Phosphorylated JNK/SAPK1 in HeLa cells



B.



Figure 1. Beadlyte® detection of phosphorylated JNK/SAPK1 proteins in HeLa cell lysate. HeLa cells were grown to 50% confluence, serum starved overnight, and heat shocked (HS) at 42°C for 30 minutes (○), or kept at 37°C (●). The cells were then incubated for 16 hours at 37°C and stimulated with (○) or without (●) 200µM arsenite (Ars) for 30 minutes. All cells were lysed in Beadlyte® Cell Signaling Lysis Buffer B with protease inhibitors. **Figure 1A** shows changes in phosphorylated JNK/SAPK1 as detected with Phospho-JNK/SAPK1 Beadmates™. Briefly, increasing amounts of cell lysate were incubated overnight at 4°C with Beadlyte® JNK/SAPK1 Beads. The Beads were washed and mixed at room temperature with Beadlyte® Anti-phospho-JNK/SAPK1, Biotin, followed by streptavidin-PE. The Median Fluorescence Intensity (MFI) was measured using the Luminex xMAP™. **Figure 1B** shows changes in phosphorylated JNK/SAPK1 as measured by Western blotting. Briefly, 20µg of cell lysate was separated by SDS-PAGE, transferred to nitrocellulose, and probed with anti-pan JNK/SAPK1 antibody followed by HRP labeled anti-IgG (for total JNK detection) or biotin labeled anti-phospho JNK/SAPK1 antibody followed by streptavidin-HRP (for phosphorylated JNK detection). Blots were visualized via chemiluminescence.

End-User License Agreement

By purchasing this product, which contains fluorescently labeled microsphere beads authorized by 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 Luminex 100. This product and the use thereof are covered by one or more of the following US patents: # 6,046,807, # 5,981,180.