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## Certificate of Analysis

**Total JNK/SAPK1 Beadmates™**  
(100 Assay Points)  
Catalog # 46-618  
Lot # 24604

### Components

**Beadlyte® Anti-JNK/SAPK1 Beads**, Catalog # 42-618, Lot # 24604. One vial containing **125µl** of anti-pan JNK/SAPK1 IgG conjugated to Luminex® Bead # 16 at **2,000 beads/µl (20X)** in a proprietary formulation of Tris-buffered salts and animal protein containing 0.05% sodium azide as a preservative.

**Beadlyte® Anti-Total-JNK/SAPK1, Biotin**, Catalog # 44-618, Lot # 24604. One vial containing **125µl** of anti-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 Kinase, both phosphorylated and non-phosphorylated forms.

**Applications:** Optimal antibody pair for detection of Total JNK/SAPK1. To be used in conjunction with Beadlyte® Cell Signaling Buffer Kits (Catalog #s 48-600 or 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**

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### Total JNK/SAPK1 Beadmate™ Description

**Use:** The Total JNK/SAPK1 Beadmate™ pair is used in conjunction with Beadlyte® Cell Signaling Buffer Kits (Catalog #s 48-600 or 48-601) to detect the presence of Total JNK/SAPK1 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>

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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 $\mu$ l) pipette + tips
- Sonication Bath (Catalog # 40-002)
- Millipore multiscreen vacuum manifold (Catalog # MAVM0960R)
- Luminex<sup>®</sup> 100™ System
- Beadlyte<sup>®</sup> 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 Total JNK/SAPK1 specific reporter, followed by streptavidin-phycoerythrin. The amount of total JNK/SAPK1 is then quantified using the Luminex<sup>®</sup> xMAP™ System.

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



*Overnight; dark  
(4 °C, shaking)*

Wash with 100 $\mu$ l Beadlyte<sup>®</sup> Cell Signaling Assay Buffer and add 25 $\mu$ l of 1X Total JNK/SAPK1 reporter solution.



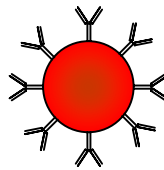
*1 hour; dark  
(RT, shaking)*

Remove reporter and add 25 $\mu$ l diluted Beadlyte<sup>®</sup> Streptavidin-Phycoerythrin.

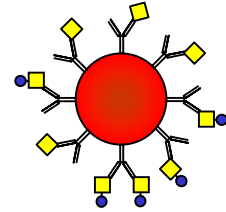


*30 min; dark  
(RT, shaking)*

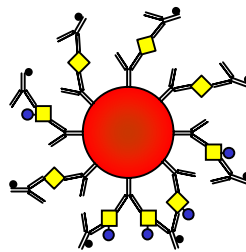
Remove Streptavidin-Phycoerythrin and resuspend in 100 $\mu$ l **Beadlyte<sup>®</sup> Cell Signaling Assay Buffer 1** and read results.



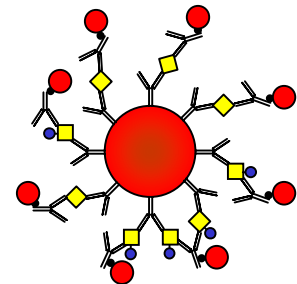
1. Bead with capture antibody



2. Capture antibody binds target proteins



3. Biotinylated reporter binds 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 Total JNK/SAPK1 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

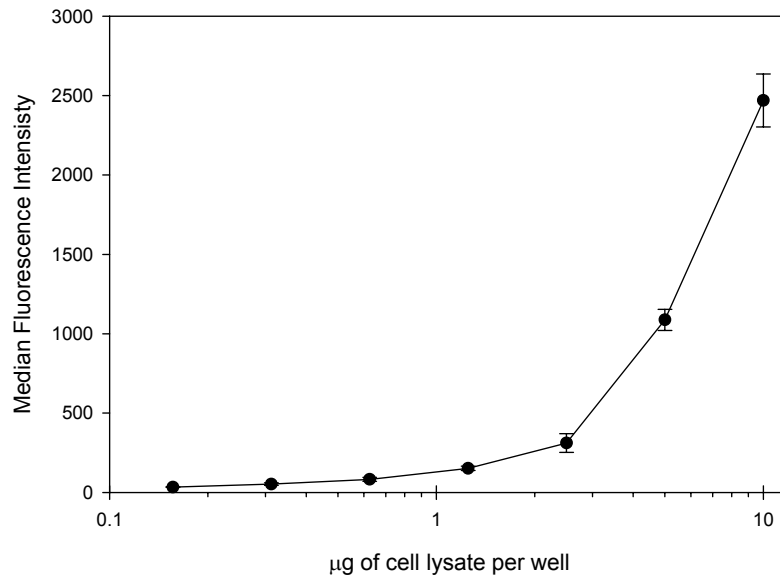
The recommended lysis and assay buffers multiplexing Total JNK/SAPK1 Beadmates™ with other Beadmates™ are Beadlyte® Cell Signaling Universal Lysis Buffer (Catalog # 43-040) and Cell Signaling Universal Assay Buffer (Catalog # 43-041). Both buffers are included in the Beadlyte® Cell Signaling Universal 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 Total JNK/SAPK1 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:

Detection of Total JNK in HeLa cells



**Figure 1. Detection of Total JNK/SAPK1 in HeLa cell lysate.** HeLa cells were grown to 50% confluence and lysed in Beadlyte<sup>®</sup> Cell Signaling Lysis Buffer B with protease inhibitors. **Figure 1** shows the levels of JNK/SAPK1 as detected with Total-JNK/SAPK1 Beadmates<sup>™</sup>. Briefly, increasing amounts of cell lysate were incubated overnight at 4°C with Beadlyte<sup>®</sup> JNK/SAPK1 Beads. The beads were washed and mixed at room temperature with Beadlyte<sup>®</sup> Anti-Total-JNK/SAPK1, Biotin, followed by streptavidin-PE. The Median Fluorescence Intensity (MFI) was measured using the Luminex<sup>®</sup> 100<sup>™</sup>.

**Figure 2. Western blot detection of Total JNK/SAPK1 in HeLa cell lysate.** 20µg of cell lysate prepared from HeLa cells lysed in Beadlyte<sup>®</sup> Cell Signaling Lysis Buffer B with protease inhibitors was separated by SDS-PAGE, transferred to nitrocellulose, and probed with anti-pan JNK/SAPK1 antibody and HRP labeled anti-IgG, and visualized via 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<sup>®</sup> 100<sup>™</sup>. This product and the use thereof are covered by one or more of the following US patents: # 6,046,807, # 5,981,180.