
Certificate of Analysis

Phospho-c-Jun (Ser73) Beadmates™
(100 Assay Points)
Catalog # 46-622
Lot # 32549

Components

Beadlyte® Anti-c-Jun Beads, Catalog # 42-622, Lot # 32549. One vial containing **125µl** of anti-c-Jun IgG conjugated to Luminex® Bead # 52 at **4,000 beads/µl (20X)** in a proprietary formulation of Tris-buffered salts and animal protein containing 0.05% sodium azide as a preservative.

Beadlyte® Anti-phospho-c-Jun (Ser73), Biotin, Catalog # 44-622, Lot # 32549. One vial containing **125µl** of anti-phospho-c-Jun 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 c-Jun phosphorylated on Ser73.

Applications: Optimal antibody pair for detection of c-Jun phosphorylated on Ser73. 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-c-Jun Beadmate™ Description

Use: The Phospho-c-Jun (Ser73) Beadmate™ pair is used in conjunction with Beadlyte® Cell Signaling Buffer Kits (Catalog #s 48-600 48-601) to detect the presence of phosphorylated c-Jun (Ser73) in cell lysates using the Luminex® 100™ 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[®] 100™ 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 c-Jun specific capture antibody overnight. The beads are washed and mixed with a biotinylated phospho-c-Jun (Ser73) specific reporter, followed by streptavidin-phycoerythrin. The amount of phospho-c-Jun is then quantified using the Luminex[®] 100™ System. A sample with unstimulated cell lysate and containing all other components will give the value for any basal phosphorylation of c-Jun.

Pre-wet filter plate and add 25 μ l of diluted cell lysate to each well with 25 μ l of 1X Jun 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-Jun reporter solution.



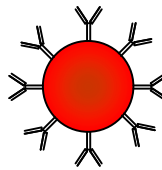
1 hour; dark
(RT, shaking)

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

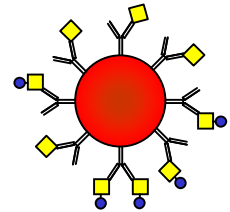


30 min; dark
(RT, shaking)

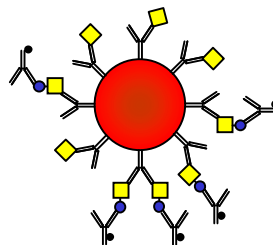
Remove Streptavidin-Phycoerythrin and resuspend in 100 μ l **Beadlyte[®] Cell Signaling Assay Buffer 1** and read results on Luminex[®] 100™ System.



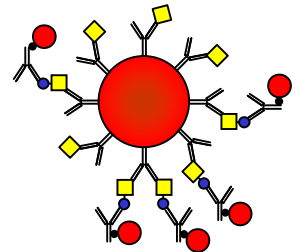
1. Bead with capture antibody



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-c-Jun (Ser73) Beadmates™ are Beadlyte® Cell Signaling Lysis Buffer C (Catalog # 43-020) and Beadlyte® Cell Signaling Assay Buffer 1 (Catalog # 43-010). 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-c-Jun (Ser73) 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-c-Jun (Ser73) 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:

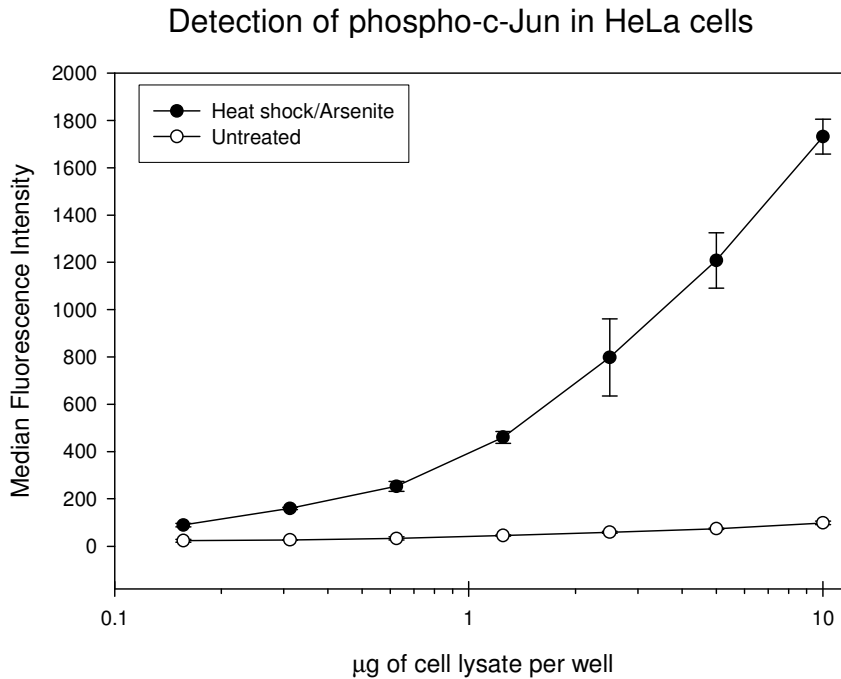
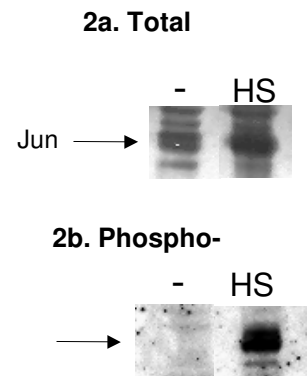


Figure 1. Beadlyte[®] detection of phosphorylated c-Jun (Ser73) in HeLa cell lysate. HeLa cells were grown to 50% confluence, serum starved overnight, and heat shocked (HS) at 42°C for 30 minutes (●), or kept at 37°C (○). The cells were then incubated for 16 hours at 37°C and stimulated with (●) or without (○) 200µM arsenite (Ars) for 30 minutes. All cells were lysed in Beadlyte[®] Cell Signaling Lysis Buffer C with protease inhibitors. **Figure 1** shows changes in phosphorylated c-Jun (Ser73) as detected with Phospho-c-Jun (Ser73) Beadmates[™]. Briefly, increasing amounts of cell lysate were incubated overnight at 4°C with Beadlyte[®] Anti-c-Jun beads. The beads were washed and mixed at room temperature with Beadlyte[®] Anti-phospho-c-Jun (Ser73), Biotin, followed by streptavidin-PE. The Median Fluorescence Intensity (MFI) was measured using the Luminex[®] 100[™] System.

Figure 2. Western blot detection of phosphorylated Jun (Ser73) in HeLa cell lysate. 20µg of lysate from heat shocked/arsenite treated (HS) or untreated cells (-) (above in Figure 1) was separated by SDS-PAGE, transferred to nitrocellulose, and probed with anti-Total c-Jun (Figure 2a) or anti-phospho-c-Jun (Ser73) (Figure 2b) antibody followed by HRP labeled anti-IgG antibody. Blots were 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[®] 100[™]. This product and the use thereof are covered by one or more of the following US patents: # 6,046,807, # 5,981,180.