



Certificate of Analysis

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Phospho-Jun (Ser73) Beadmates™
(100 Assay Points)
Catalog # 46-622
Lot # 27755

Components

Beadlyte® Anti-Jun Beads, Catalog # 42-622, Lot # 27755. One vial containing **125µl** of anti-Jun IgG conjugated to Luminex® Bead # 52 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-phospho-Jun (Ser73), Biotin, Catalog # 44-622, Lot # 27755. One vial containing **125µl** of anti-phospho-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 Jun phosphorylated on Ser73.

Applications: Optimal antibody pair for detection of Jun phosphorylated on Ser73. To be used in conjunction with the Beadlyte® Cell Signaling Buffer Kit (Catalog # 48-600).

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-Jun Beadmate™ Description

Use: The Phospho-Jun Beadmate™ pair is used in conjunction with the Beadlyte® Cell Signaling Buffer Kit (Catalog # 48-600) to detect the presence of phosphorylated 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. The Beadlyte® Cell Signaling Buffer Kit is also ordered separately and consists 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 for the most recent (highest number) lot of the Beadlyte® Cell Signaling Buffer Kit available at:

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

(This page can also be accessed from <http://www.beadlyte.com> by entering **48-600** in the search box.)

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)

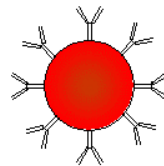
Detection Protocol Summary

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 Jun specific capture antibody overnight. The beads are washed and mixed with a biotinylated phospho-Jun (Ser73) specific reporter, followed by streptavidin-phycoerythrin. The amount of phospho-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 Jun.

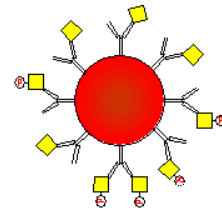
Pre-wet filter plate and add 25 μ l of diluted cell lysate to each well with 25 μ l of 1X Jun bead solution.



Overnight; dark
(4°C, shaking)



1. Bead with capture antibody

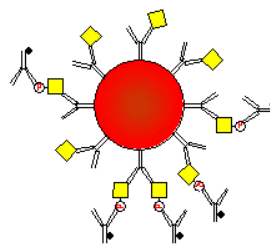


2. Capture antibody binds target proteins

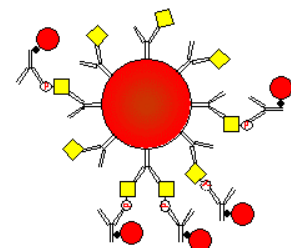
Wash with 100 μ l Beadlyte[®] Cell Signaling Assay Buffer and add 25 μ l of 1X Phospho-Jun reporter solution.



1 hour; dark
(RT, shaking)



3. Biotinylated reporter binds phosphorylated proteins



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

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.

Recommendations for Protocol

Preparation of lysates

For a single plex analysis, Beadlyte[®] Cell Signaling **Lysis Buffer C** is recommended for lysing cells for Phospho-Jun single plex analysis. This lysate buffer is included in the Beadlyte[®] Cell Signaling Buffer Kit (Catalog # 48-600). Refer to the Beadlyte[®] Cell Signaling Buffer Kit COA (select the highest lot number) for a suggested cell lysis protocol at:

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

Note: If the cell lysate is to be used in a multiplex assay with Phospho-Jun beads and other Beadmates[™], please refer to the Buffer Selection Table in the Beadlyte[®] Cell Signaling Buffer Kit COA (select the highest lot number) at <http://www.upstate.com/browse/productdetail.asp?ProductId=48-600> to select the best Lysis Buffer.

Preparation of Phospho-Jun Beads and reporter antibodies

For Phospho-Jun single plex analysis, Beadlyte[®] Cell Signaling **Assay Buffer 1** is recommended for best results (Beadlyte[®] Cell Signaling Buffer Kit, Catalog # 48-600).

Note: If Phospho-Jun beads are being multiplexed with other Beadmates[™], please refer to the Buffer Selection Table in the Beadlyte[®] Cell Signaling Buffer Kit COA (select the highest lot number) at <http://www.upstate.com/browse/productdetail.asp?ProductId=48-600> to select the best Assay Buffer to use.

Phospho-Jun Buffer Selection Chart

Beadmate	Catalog #	Bead #	Lysis buffer	Assay buffer 1 activity (%)	Assay buffer 2 activity (%)	Assay buffer 3 activity (%)
Phospho-Jun	46-622	52	A	0-20	0-20	0-20
			B	80-100	20-40	20-40
			C	100	20-40	20-40

Representative Data:

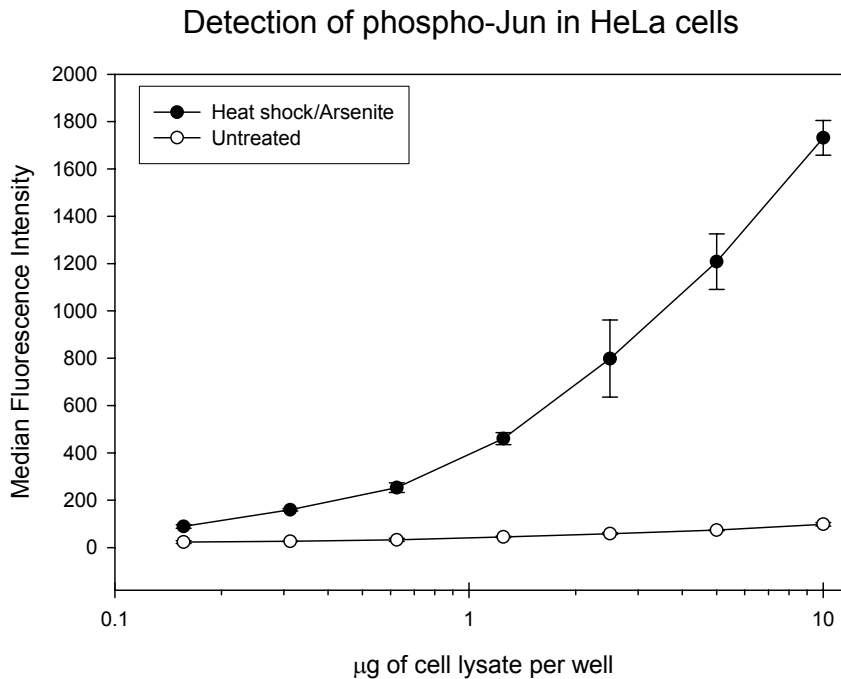
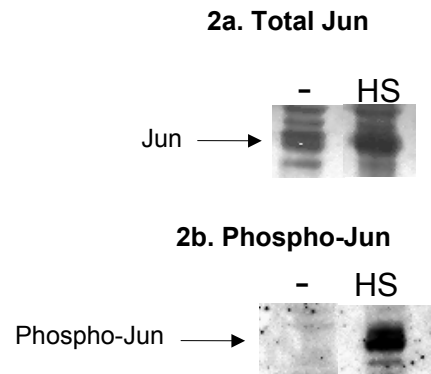


Figure 1. Beadlyte[®] detection of phosphorylated Jun (Ser73) in HeLa cell lysate. HeLa cells were grown to 50% confluence, serum starved overnight, and heat shocked (HS) at 42°C for 30 minutes (●), or kept at 37°C (○). The cells were then incubated for 16 hours at 37°C and stimulated with (●) or without (○) 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 Jun (Ser 73) as detected with Phospho-Jun (Ser73) Beadmates[™]. Briefly, increasing amounts of cell lysate were incubated overnight at 4°C with Beadlyte[®] Anti-Jun beads. The beads were washed and mixed at room temperature with Beadlyte[®] Anti-phospho-Jun (Ser 73), 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 Jun (Figure 2a) or anti-phospho Jun (Ser 73) (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.