
Certificate of Analysis

Total JNK/SAPK1 Beadmates™
(100 Assay Points)
Catalog # 46-618
Lot # 33412

Components

Beadlyte® Anti-JNK/SAPK1 Beads, Catalog # 42-618, Lot # 33412. One vial containing **125µl** of anti-pan JNK/SAPK1 IgG conjugated to Luminex® Bead # 16 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-JNK/SAPK1, Biotin, Catalog # 44-618, Lot # 33412. 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 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

Total JNK/SAPK1 Beadmate™ Description

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

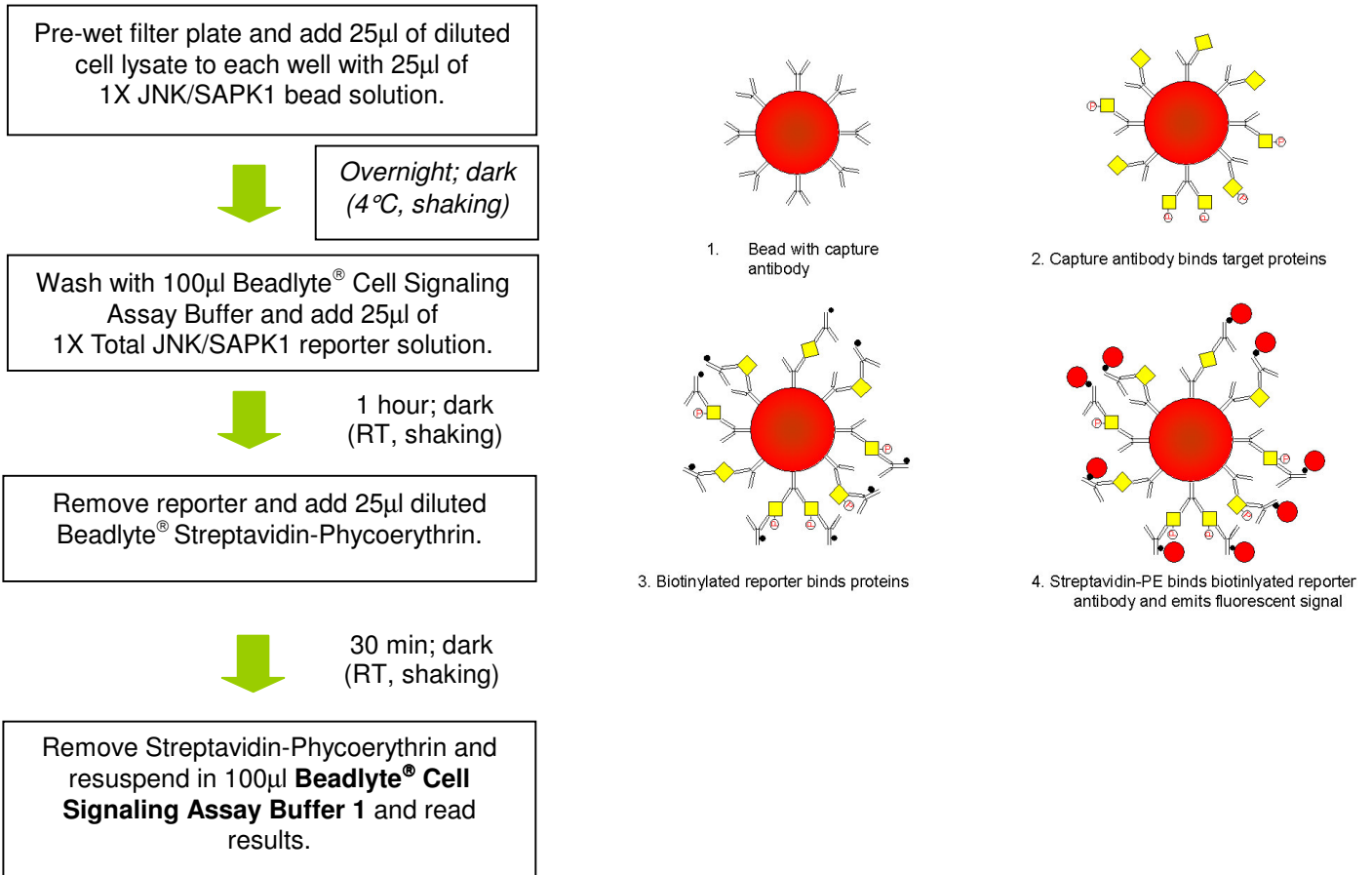
(This page can also be accessed from <http://www.beadlyte.com> by entering **48-600** in the search box.)

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 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[®] 100[™] System.



Recommendations for Protocol

Preparation of Lysates

For a single plex analysis, Beadlyte[®] Cell Signaling **Lysis Buffer B** is recommended for lysing cells for Total JNK/SAPK1 single plex analysis. This lysate buffer is included in the Beadlyte[®] Cell Signaling Buffer Kit (Catalog # 48-600). Refer to the most recent (highest number) Beadlyte[®] Cell Signaling Buffer Kit COA for a suggested cell lysis protocol 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.

Note: If the cell lysate is to be used in a multiplex assay with Total JNK/SAPK1 beads and other Beadmates[™], please refer to the [Buffer Selection Table](#) in the Beadlyte[®] Cell Signaling Buffer Kit COA at the Beadlyte[®] website described above to select the best Lysis Buffer.

Preparation of Total JNK/SAPK1 Beads and Reporter Antibodies

For Total JNK/SAPK1 single plex analysis, Beadlyte[®] Cell Signaling **Assay Buffer 2** is recommended for best results (Beadlyte[®] Cell Signaling Buffer Kit, Catalog # 48-600).

Note: If Total JNK/SAPK1 beads are being multiplexed with other Beadmates[™], please refer to the [Buffer Selection Table](#) in the Beadlyte[®] Cell Signaling Buffer Kit COA at the Beadlyte[®] website described above to select the best Assay Buffer to use.

Total JNK/SAPK1 Buffer Selection Chart

Beadmate	Catalog #	Bead #	Lysis buffer	Assay buffer 1 activity (%)	Assay buffer 2 activity (%)	Assay buffer 3 activity (%)
Total JNK/SAPK1	46-618	16	A	0	20-40	0
			B	0-20	100	0-20
			C	0	20-40	0

Representative Data:

Detection of Total JNK in HeLa cells

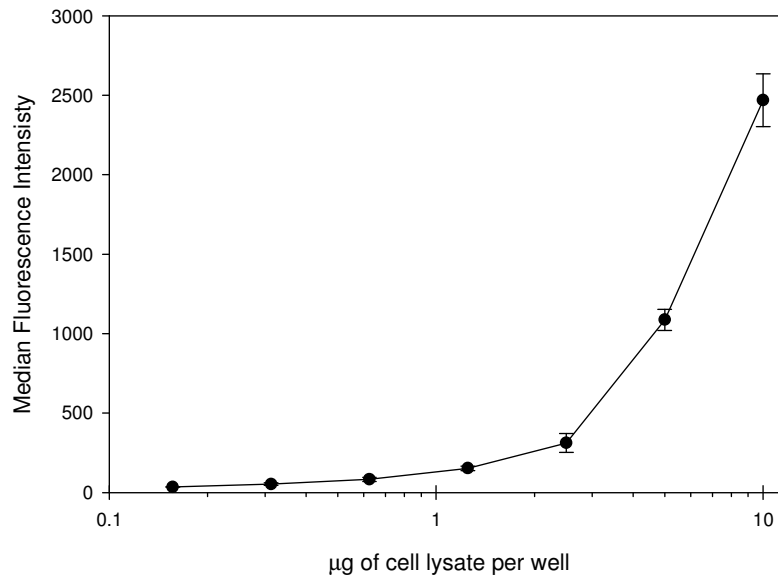


Figure 1. Detection of Total JNK/SAPK1 in HeLa cell lysate. HeLa cells were grown to 50% confluence and lysed in Beadlyte[®] Cell Signaling Lysis Buffer B with protease inhibitors. **Figure 1** shows the levels of JNK/SAPK1 as detected with Total-JNK/SAPK1 Beadmates[™]. Briefly, increasing amounts of 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-Total-JNK/SAPK1, Biotin, followed by streptavidin-PE. The Median Fluorescence Intensity (MFI) was measured using the Luminex[®] 100[™].

Figure 2. Western blot detection of Total JNK/SAPK1 in HeLa cell lysate. 20µg of cell lysate prepared from HeLa cells lysed in Beadlyte[®] 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[®] 100[™]. This product and the use thereof are covered by one or more of the following US patents: # 6,046,807, # 5,981,180.