



cell signaling solutions

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*Certificate of Analysis*

10 Old Barn Road • Lake Placid, NY 12946  
Technical Support: T: 800 548-7853 • F: 518 523-4513  
email: techserv@upstate.com  
Sales Department: T: 800 233-3991 • F: 781 890-7738  
Licensing Dept.: 800 310-4659  
www.upstate.com

## Phospho-HSP27 (Ser78) Beadmates™

(100 Assay Points)

Catalog # 46-607

Lot # 26453A

### Components

**Beadlyte® Anti-HSP27 Beads**, Catalog # 42-607, Lot # 26453. One vial containing **125µl** of anti-HSP27 IgG conjugated to Luminex® Bead #50 at **2000 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-HSP27 (Ser78), Biotin**, Catalog # 44-607, Lot # 26453A. One vial containing **125µl** of biotin-conjugated anti-phospho-HSP27 (Ser78) IgG (**20X**) in a proprietary formulation of Tris-buffered salts and animal protein containing 0.05% sodium azide as a preservative.

**Specificity:** Recognizes human HSP27 phosphorylated on Ser78.

**Applications:** Optimal antibody pair for detection of HSP27 phosphorylated on Ser78. 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**

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### Phospho-HSP27 Beadmate™ Description

**Use:** The Phospho-HSP27 Beadmate™ pair is used in conjunction with the Beadlyte® Cell Signaling Buffer Kit (Catalog # 48-600) to detect the presence of phosphorylated HSP27 (Ser78) in cell lysates using the Luminex 100 xMAP 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 (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 xMAP™ System
- Beadlyte® Cell Signaling Buffer Kit (Catalog # 48-600)

### Detection Protocol Summary

The assay procedure is a fluorescent bead-based sandwich immunoassay that is sensitive and easy to perform. A cell lysate or other sample is incubated with beads coupled to an HSP27 specific antibody. The beads are washed and mixed with a biotinylated phospho-HSP27 specific reporter, followed by streptavidin-phycoerythrin. The amount of phospho-HSP27 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 HSP27.

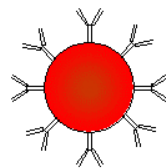
**Important note:** For a detailed protocol on Cell Signaling Detection Procedures please see the COA (select the highest lot number) for the Beadlyte® Cell Signaling Buffer Kit available at:

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

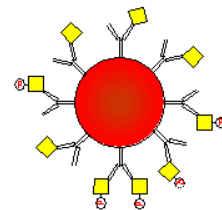
Pre-wet filter plate and add 25 $\mu$ l of diluted cell lysate to each well with 25 $\mu$ l of 1X HSP27 bead solution.



*Overnight; dark  
(4 °C, shaking)*



1. Bead with capture antibody

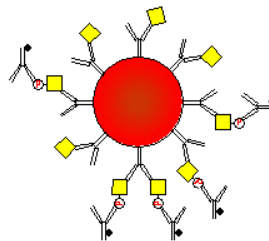


2. Capture antibody binds target proteins

Wash with 100 $\mu$ l Beadlyte® Cell Signaling Assay Buffer and add 25 $\mu$ l of 1X Phospho-HSP27 reporter solution.



*1 hour; dark  
(RT, shaking)*

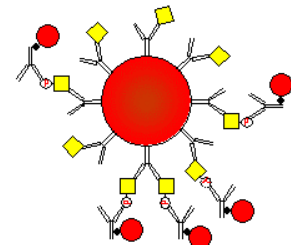


3. Biotinylated reporter binds phosphorylated proteins

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



*30 min; dark  
(RT, shaking)*



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

Remove Streptavidin-Phycoerythrin and resuspend in 100 $\mu$ l **Beadlyte® Cell Signaling Assay Buffer 1** and read results on Luminex xMAP™.

## Recommendations for Protocol

### Preparation of lysates

Beadlyte<sup>®</sup> Cell Signaling **Lysis Buffer B** is recommended to lyse cells for Phospho-HSP27 single-plex analysis. This lysis buffer is included in the Beadlyte<sup>®</sup> Cell Signaling Buffer Kit (Catalog # 48-600). Refer to the Beadlyte<sup>®</sup> 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-HSP27 beads and other Beadmates<sup>™</sup>, please refer to the Buffer Selection Table in the Beadlyte<sup>®</sup> 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-HSP27 Beads and reporter antibodies

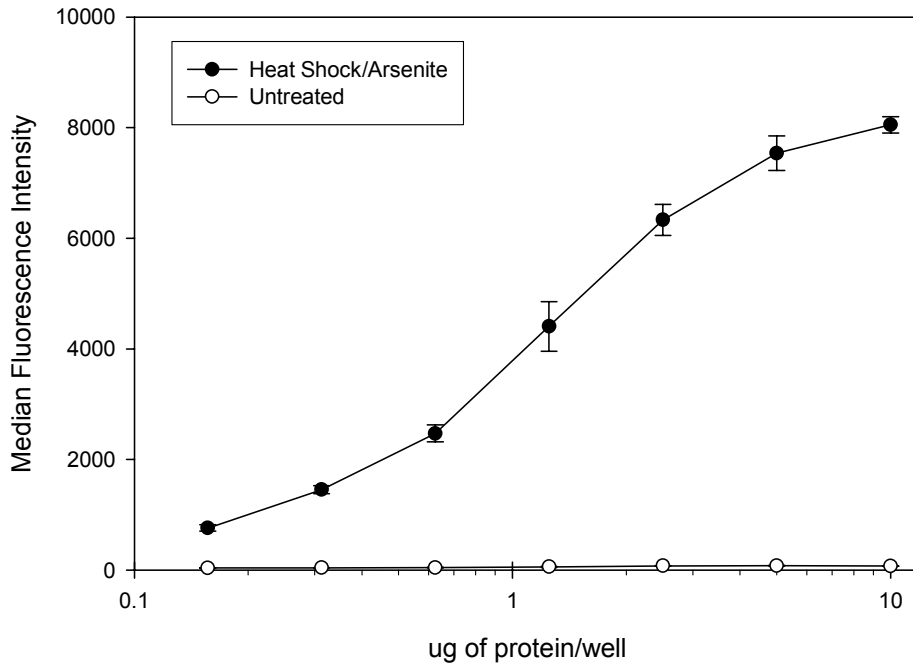
For Phospho-HSP27 single-plex analysis, Beadlyte<sup>®</sup> Cell Signaling **Assay Buffer 1** is recommended for best results (Beadlyte<sup>®</sup> Cell Signaling Buffer Kit, Catalog # 48-600).

**Note:** If Phospho-HSP27 beads are being multiplexed with other Beadmates<sup>™</sup>, please refer to the Buffer Selection Table in the Beadlyte<sup>®</sup> 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-HSP27 Buffer Selection Chart

Beadmate	Catalog #	Bead #	Lysis buffer	Assay buffer 1 activity (%)	Assay buffer 2 activity (%)	Assay buffer 3 activity (%)
Phospho-HSP27	46-607	#50	A	60-80	40-60	60-80
			B	<b>100</b>	60-80	60-80
			C	60-80	40-60	60-80

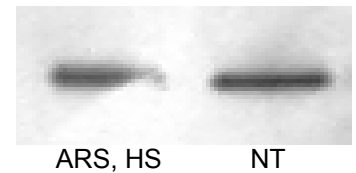
**Representative Data:**



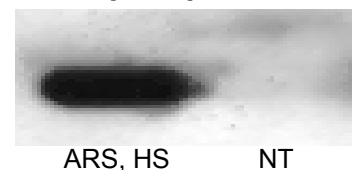
**Figure 1. Beadlyte<sup>®</sup> detection of phosphorylated HSP27 proteins in HeLa cell lysate.** HeLa cells were grown to 50% confluence and the cells to be stimulated were heat shocked at 42°C for 30 minutes, while the unstimulated cells were kept at 37°C. The cells were then grown at 37°C for 16 hours (70% confluence) and stimulated with (●) or without (○) 200µM arsenite for 30 minutes, washed twice with TBS and lysed in Beadlyte<sup>®</sup> Cell Signaling Lysis Buffer A with protease inhibitors. Increasing amounts of cell lysate were incubated overnight at 4°C with Beadlyte<sup>®</sup> Anti-HSP27 Beads. The Beads were washed and mixed at room temperature with Beadlyte<sup>®</sup> Anti-Phospho-HSP27 (Ser78), Biotin, followed by streptavidin-PE. The Median Fluorescence Intensity (MFI) was measured using the Luminex xMAP<sup>™</sup> system.

**Figure 2. Western blot detection of total and phospho-HSP27 in HeLa cell lysate.** HeLa cells were grown to 50% confluence and the cells to be stimulated were heat shocked at 42°C for 30 minutes, while the unstimulated cells were kept at 37°C. The cells were then grown at 37°C for 16 hours (70% confluence) and stimulated with (HS, ARS) or without (NT) 200µM arsenite for 30 minutes. 10µg of total lysate proteins per well (lysed in Beadlyte<sup>®</sup> Cell Signaling Lysis Buffer A with protease inhibitors) were separated by SDS-PAGE, transferred to nitrocellulose, and probed with anti-HSP27 (fig. 2a) or anti-phospho-HSP27 (Ser78), (fig. 2b). Blots were incubated with an HRP-labeled secondary antibody, and visualized via chemiluminescence.

**2a. anti-HSP27**



**2b. anti-phospho-HSP27**



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