

**Phospho-HSP27 (Ser78) Beadmates™**

(100 Assay Points)

Catalog # 46-607

Lot # 27443

**Components**

**Beadlyte® Anti-HSP27 Beads**, Catalog # 42-607, Lot # 27443. 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 # 27443. 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**

---

**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™ 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>

---

Other components required but not included as part of kit are:

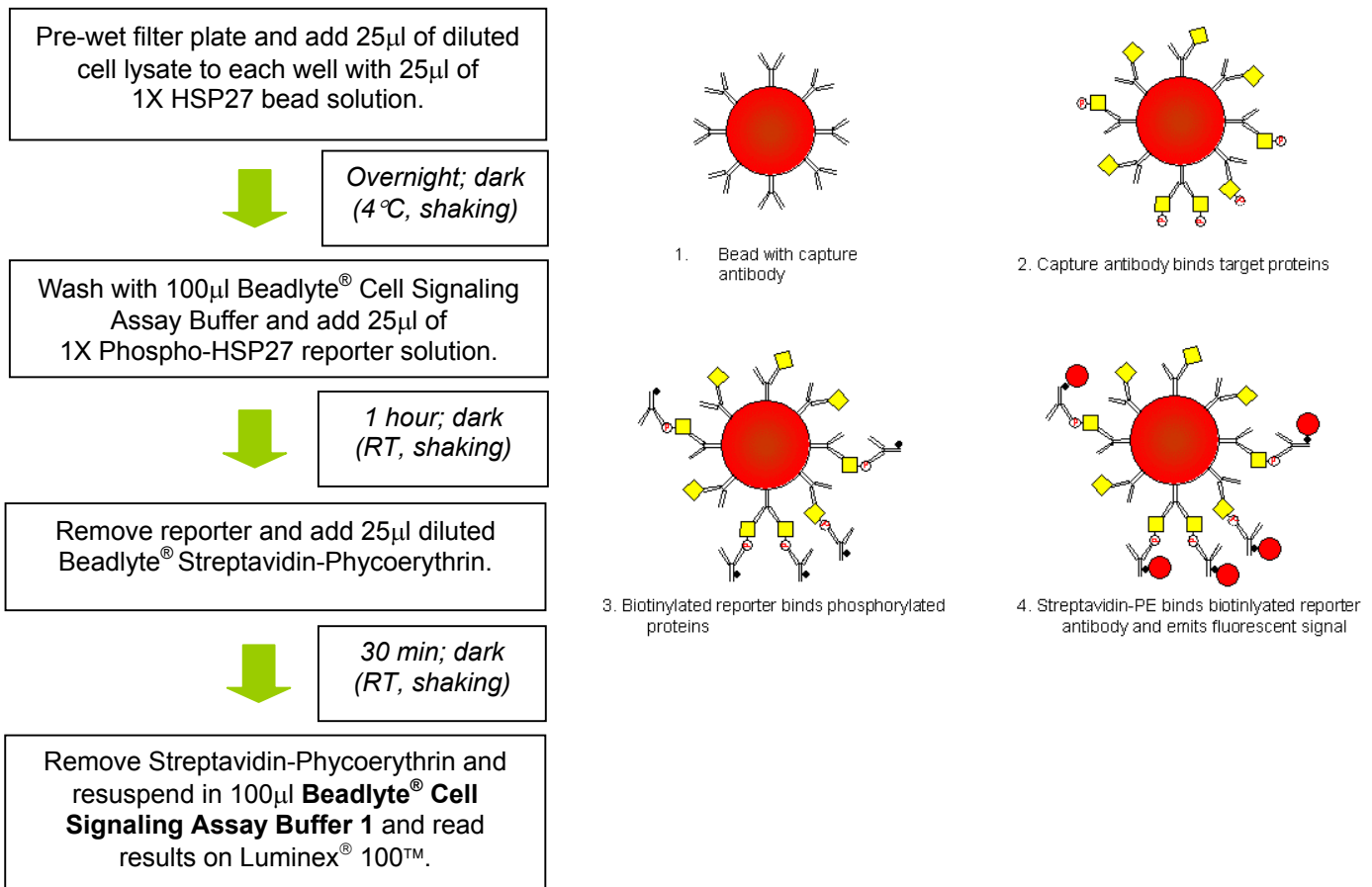
- Cell lysates or cell extracts harboring protein(s) of interest
- Vortex mixer
- Plate shaker
- Timer
- Variable volume (5-200 $\mu$ l) pipette + tips
- Sonication Bath (Catalog # 40-002)
- Millipore multiscreen vacuum manifold (Catalog # MAVM0960R)
- Luminex<sup>®</sup> 100™ System
- Beadlyte<sup>®</sup> Cell Signaling Buffer Kit (Catalog # 48-600)

### 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<sup>®</sup> 100™ 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<sup>®</sup> Cell Signaling Buffer Kit available at:

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



## 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

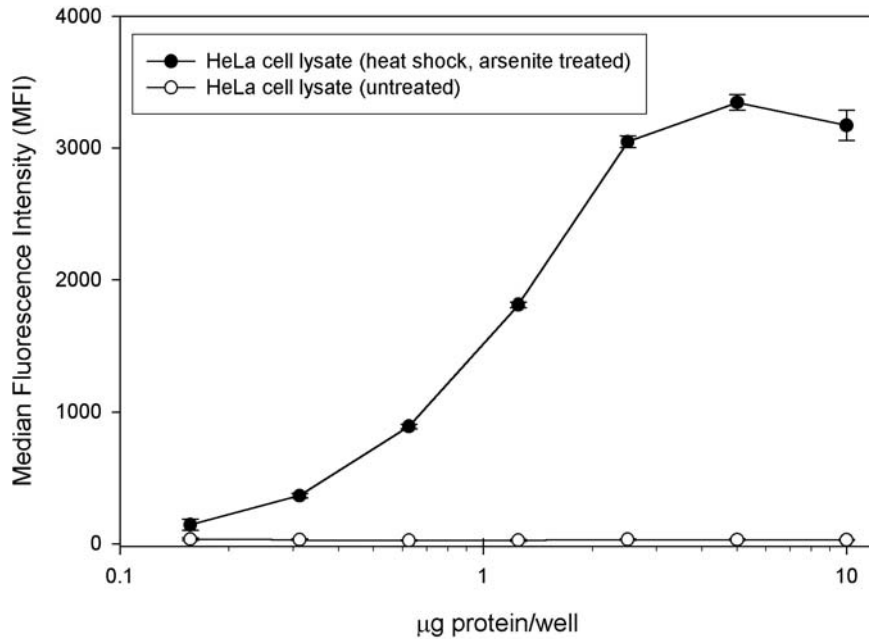
For Phospho-HSP27 single-plex analysis, Beadlyte<sup>®</sup> Cell Signaling **Assay Buffer 1** is recommended for best results (Beadlyte<sup>®</sup> Cell Signaling Buffer Kit, Catalog # 48-600).

**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

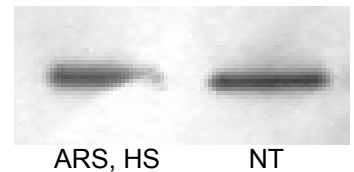
**Representative Data:**



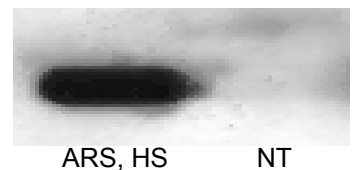
**Figure 1. Beadlyte® detection of phosphorylated HSP27 proteins in HeLa cell lysate.** HeLa cells were grown to 50% confluence and the cells to be stimulated were heat shocked at 42°C for 30 minutes, while the unstimulated cells were kept at 37°C. The cells were then grown at 37°C for 16 hours (70% confluence) and stimulated with (●) or without (○) 200µM arsenite for 30 minutes, washed twice with TBS and lysed in Beadlyte® Cell Signaling Lysis Buffer A with protease inhibitors. Increasing amounts of cell lysate were incubated overnight at 4°C with Beadlyte® Anti-HSP27 Beads. The Beads were washed and mixed at room temperature with Beadlyte® Anti-Phospho-HSP27 (Ser78), Biotin, followed by streptavidin-PE. The Median Fluorescence Intensity (MFI) was measured using the Luminex® 100™ 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® 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**



**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™. One or more of the following US patents covers this product and the use thereof: #6,046,807, #5,981,180.