
Certificate of Analysis

Phospho-HSP27 (Ser78) Beadmates™
(100 Assay Points)
Catalog # 46-607
Lot # 32402

Components

Beadlyte® Anti-HSP27 Beads, Catalog # 42-607, Lot # 32402. One vial containing **125µl** of anti-HSP27 IgG conjugated to Luminex® Bead #50 at **4000 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 # 32402. 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 Beadlyte® Cell Signaling Buffer Kits (Catalog #s 48-600 or 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-HSP27 Beadmate™ Description

Use: The Phospho-HSP27 (Ser78) Beadmate™ pair is used in conjunction with Beadlyte® Cell Signaling Buffer Kits (Catalog #s 48-600 or 48-601) 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. 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 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[®] 100[™] System. A sample with unstimulated cell lysate and containing all other components will give the value for any basal phosphorylation of HSP27.

Pre-wet filter plate and add 25 μ l of diluted cell lysate to each well with 25 μ l of 1X HSP27 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-HSP27 reporter solution.



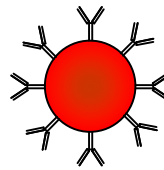
*1 hour; dark
(RT, shaking)*

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

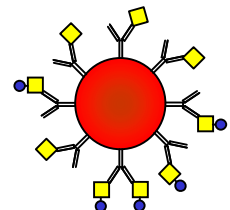


*30 min; dark
(RT, shaking)*

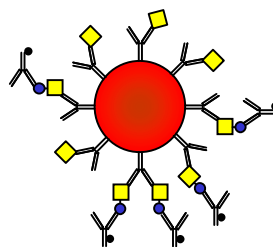
Remove Streptavidin-Phycoerythrin and resuspend in 100 μ l **Beadlyte[®] Cell Signaling Assay Buffer 1** and read results on Luminex[®] 100[™].



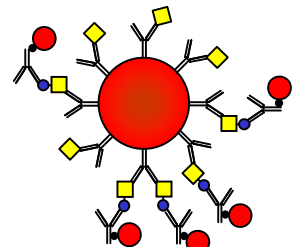
1. Bead with capture antibody



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-HSP27 (Ser78) Beadmates™ are Beadlyte® Cell Signaling **Lysis Buffer B** (Catalog # 43-019) 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-HSP27 (Ser78) 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-HSP27 (Ser78) 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:

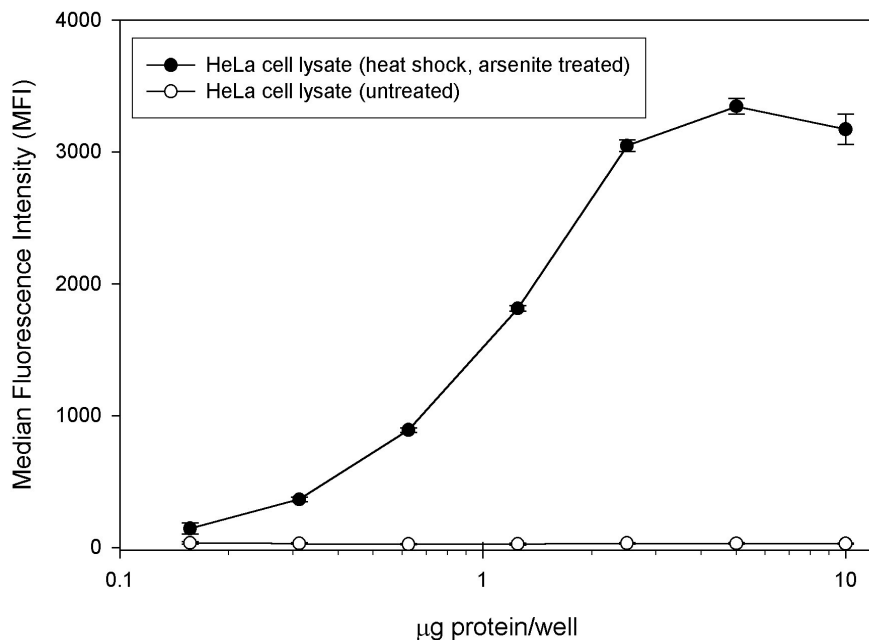
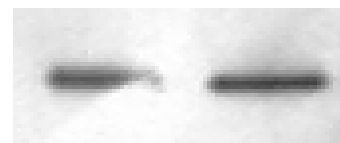


Figure 1. Beadlyte® detection of phosphorylated HSP27 proteins in HeLa cell lysate. HeLa cells were grown to 50% confluence and the cells to be stimulated were heat shocked at 42°C for 30 minutes, while the unstimulated cells were kept at 37°C. The cells were then grown at 37°C for 16 hours (70% confluence) 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.

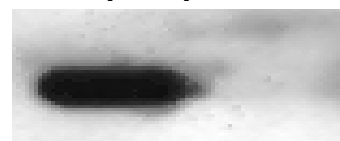
Figure 2. Western blot detection of total and phospho-HSP27 in HeLa cell lysate. HeLa cells were grown to 50% confluence and the cells to be stimulated were heat shocked at 42°C for 30 minutes, while the unstimulated cells were kept at 37°C. The cells were then grown at 37°C for 16 hours (70% confluence) 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



ARS, HS NT

2b. anti-phospho-HSP27



ARS, HS NT

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.