



cell signaling solutions

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## Certificate of Analysis

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**Beadlyte<sup>®</sup> Total HSP27 Beadmates<sup>™</sup>**  
(100 Assay Points)  
Catalog # 46-608  
Lot # 22498

### Components

**Beadlyte<sup>®</sup> Total HSP27 Beads**, Catalog # 42-608, Lot # 22498. One vial containing **125 $\mu$ l** of anti-HSP27 mouse monoclonal IgG conjugated to Luminex<sup>™</sup> Bead #50 at **2000 beads/ $\mu$ l (20X)** in a proprietary formulation of Tris buffered salts and animal protein containing 0.05% sodium azide as a preservative.

**Beadlyte<sup>®</sup> Biotinylated Total HSP27 Reporter**, Catalog # 44-608, Lot # 22498. One vial containing **125 $\mu$ l** of anti-HSP27 rabbit 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, both phosphorylated and non-phosphorylated forms.

**Applications:** Optimal antibody pair for detection of HSP27. To be used in conjunction with the Beadlyte<sup>®</sup> 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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### Total HSP27 Beadmate<sup>™</sup> Description

**Use:** The Beadlyte<sup>®</sup> Total HSP27 Beadmate<sup>™</sup> pair is used in conjunction with the Beadlyte<sup>®</sup> Cell Signaling Buffer Kit (Catalog # 48-600) to detect the presence of total HSP27 in cell lysates using the Luminex<sup>100</sup> system. Each Beadmate<sup>™</sup> pair is ordered individually and can be combined for simultaneous multiplex analysis of cellular events. The Beadlyte<sup>®</sup> Cell Signaling Buffer Kit is also ordered separately and consists of a common set of reagents needed for using Beadmates<sup>™</sup>. 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:** The Beadlyte<sup>®</sup> Total HSP27Beadmate<sup>™</sup> pair CANNOT be multiplexed with the Beadlyte<sup>®</sup> Phospho-HSP27Beadmate<sup>™</sup> pair (Catalog # 46-607) since it would require a second reporter fluorochrome on one of the antibodies. The current Luminex systems are **not** able to perform two color analysis at this time. For a detailed protocol on Cell Signaling Detection Procedures please see the COA for the Beadlyte<sup>®</sup> Cell Signaling Buffer Kit available at:

<http://www.upstate.com/img/coa/48-600-24907.pdf>

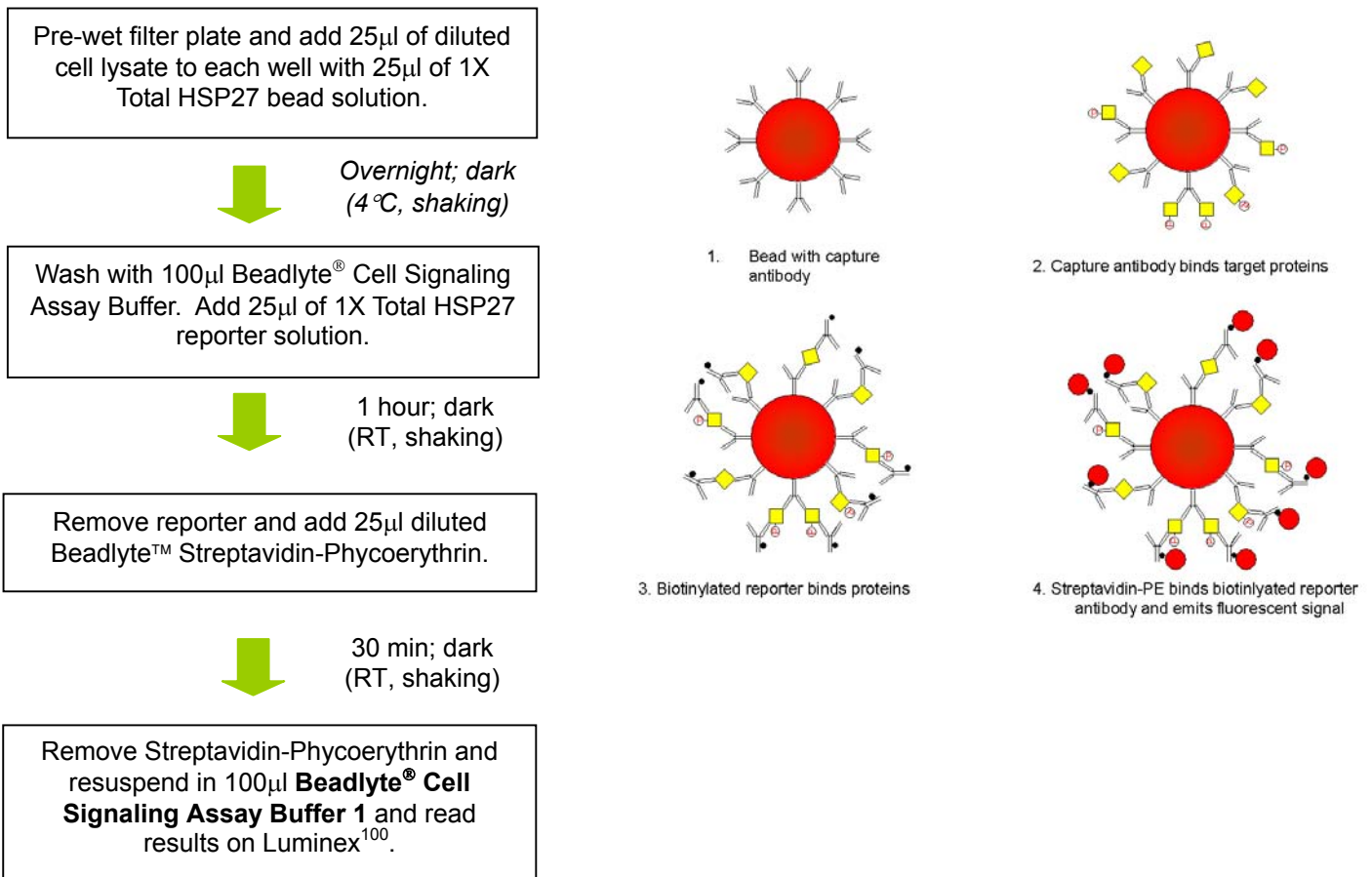
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Other components required but not included as part of kit are:

- 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<sup>100</sup> LabMAP™ 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 overnight with beads coupled to a capture antibody that binds HSP27 at a site distinct from the secondary reporter site, capturing both phosphorylated and non-phosphorylated forms of HSP27. The beads are washed and mixed with a biotinylated total HSP27 specific reporter, followed by streptavidin-phycoerythrin. The amount of total HSP27 is then quantified using the Luminex<sup>100</sup> LabMAP™ System.



## Recommendations for Protocol

### Preparation of lysates

For a single plex analysis, Beadlyte<sup>®</sup> Cell Signaling **Lysis Buffer B** is recommended for lysing cells for Total HSP27 analysis. This lysate 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 for a suggested cell lysis protocol at: <http://www.upstate.com/img/coa/48-600-24907.pdf>.

**Note:** If the cell lysate is to be multiplexed with Total HSP27 beads and other Beadmates<sup>™</sup>, please refer to the Buffer Selection Table in the Beadlyte<sup>®</sup> Cell Signaling Buffer Kit COA at <http://www.upstate.com/img/coa/48-600-24907.pdf> to select the best Lysis Buffer.

### Preparation of Total HSP27 Beads and reporter antibodies

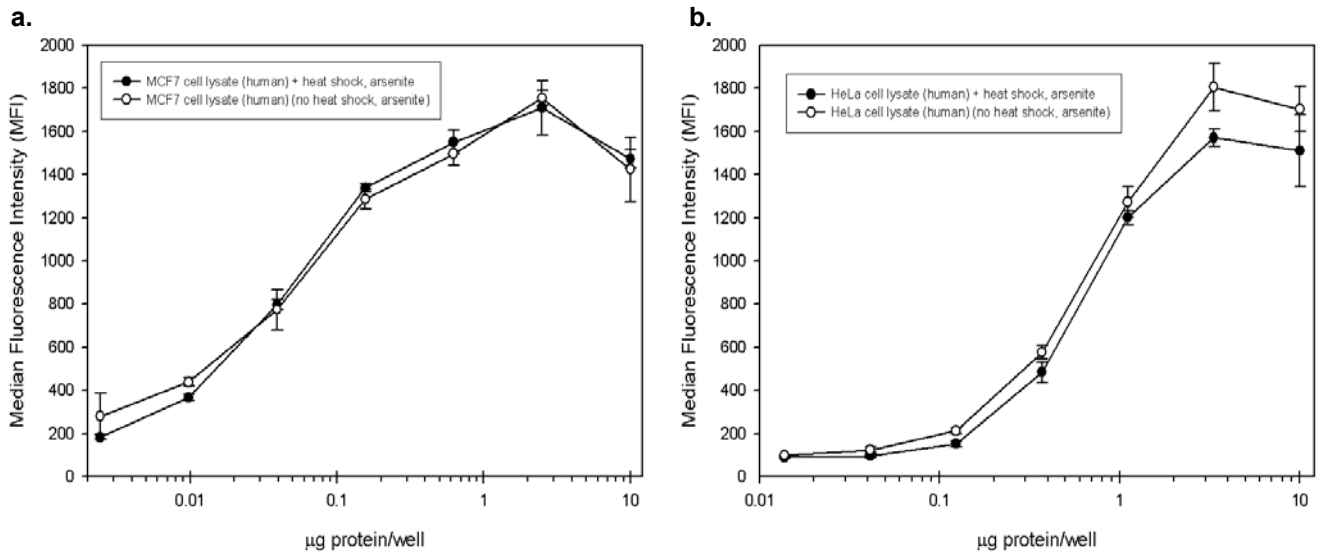
For Total HSP27 single plex analysis, Beadlyte<sup>®</sup> Cell Signaling **Assay Buffer 3** is recommended for best results (Beadlyte<sup>®</sup> Cell Signaling Buffer Kit, Catalog # 48-600).

**Note:** If Total 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 at <http://www.upstate.com/img/coa/48-600-24907.pdf> to select the best Assay Buffer to use.

### Total HSP27 Buffer Selection Chart

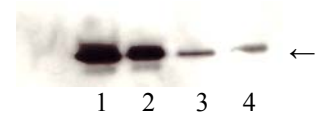
Beadmate <sup>™</sup>	Catalog #	Bead 3	Lysis buffer	Assay buffer 1 activity (%)	Assay buffer 2 activity (%)	Assay buffer 3 activity (%)
Total HSP27	46-608	#50	A	60-80	20-40	60-80
			B	80-100	40-60	<b>100</b>
			C	80-100	20-40	60-80

**Representative Data:**



**Figure 1. Beadlyte<sup>®</sup> detection of total HSP27 proteins in MCF7 cell lysate (a) and HeLa cell lysate (b).** MCF7 cells (a) were grown to 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 and stimulated with (●) or without (○) 200µM arsenite for 30 minutes. HeLa cells (b) were grown in the same manner. Increasing amounts of cell lysate (lysed in Beadlyte<sup>®</sup> Lysis Buffer A with protease inhibitors) were incubated overnight at 4°C with Beadlyte<sup>®</sup> Total HSP27 Beads. The Beads were washed and mixed at room temperature with Beadlyte<sup>®</sup> Total HSP27 Reporter, followed by streptavidin-PE. The Median Fluorescence Intensity (MFI) was measured using the Luminex<sup>100</sup> LabMAP™ system.

**Figure 2. Western blot detection of total HSP27 in MCF7 cell lysate and HeLa cell lysate.** MCF7 cells were grown to 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 and stimulated with or without 200µM arsenite for 30 minutes. HeLa cells were grown in the same manner. 10µg/well of unstimulated MCF7 (lane 1), stimulated MCF7 (lane 2), unstimulated HeLa (lane 3) or stimulated HeLa (lane 4) cell lysate (lysed in Beadlyte<sup>®</sup> Lysis Buffer A with protease inhibitors) were separated by SDS-PAGE, transferred to nitrocellulose, and probed with rabbit anti-HSP27. Blots were incubated with HRP labeled anti-rabbit IgG and visualized via chemiluminescence. Arrow indicates HSP27 (27kDa).



**End-User License Agreement**

By purchasing this product, which contains fluorescently labeled microsphere beads authorized by Luminex™, you, the customer, acquire the right under Luminex'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<sup>100</sup>. This product and the use thereof are covered by one or more of the following US patents: # 6,046,807, # 5,981,180.