
Certificate of Analysis

Total HSP27 Beadmates™

(100 Assay Points)

Catalog # 46-608

Lot # 0608038812

Components

Beadlyte® Anti-HSP27 Beads, Catalog # 42-608, Lot # 0608038812. One vial containing **125µl** of anti-HSP27 mouse monoclonal 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-Total HSP27, Biotin, Catalog # 44-608, Lot # 0608038812. One vial containing **125µl** 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® 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 HSP27 Beadmate™ Description

Use: The Total HSP27 Beadmate™ pair is used in conjunction with the Beadlyte® Cell Signaling Buffer Kit (Catalog # 48-600) to detect the presence of total HSP27 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: The Total HSP27 Beadmate™ pair CANNOT be multiplexed with the Phospho-HSP27 Beadmate™ 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® Cell Signaling Buffer Kit available at:

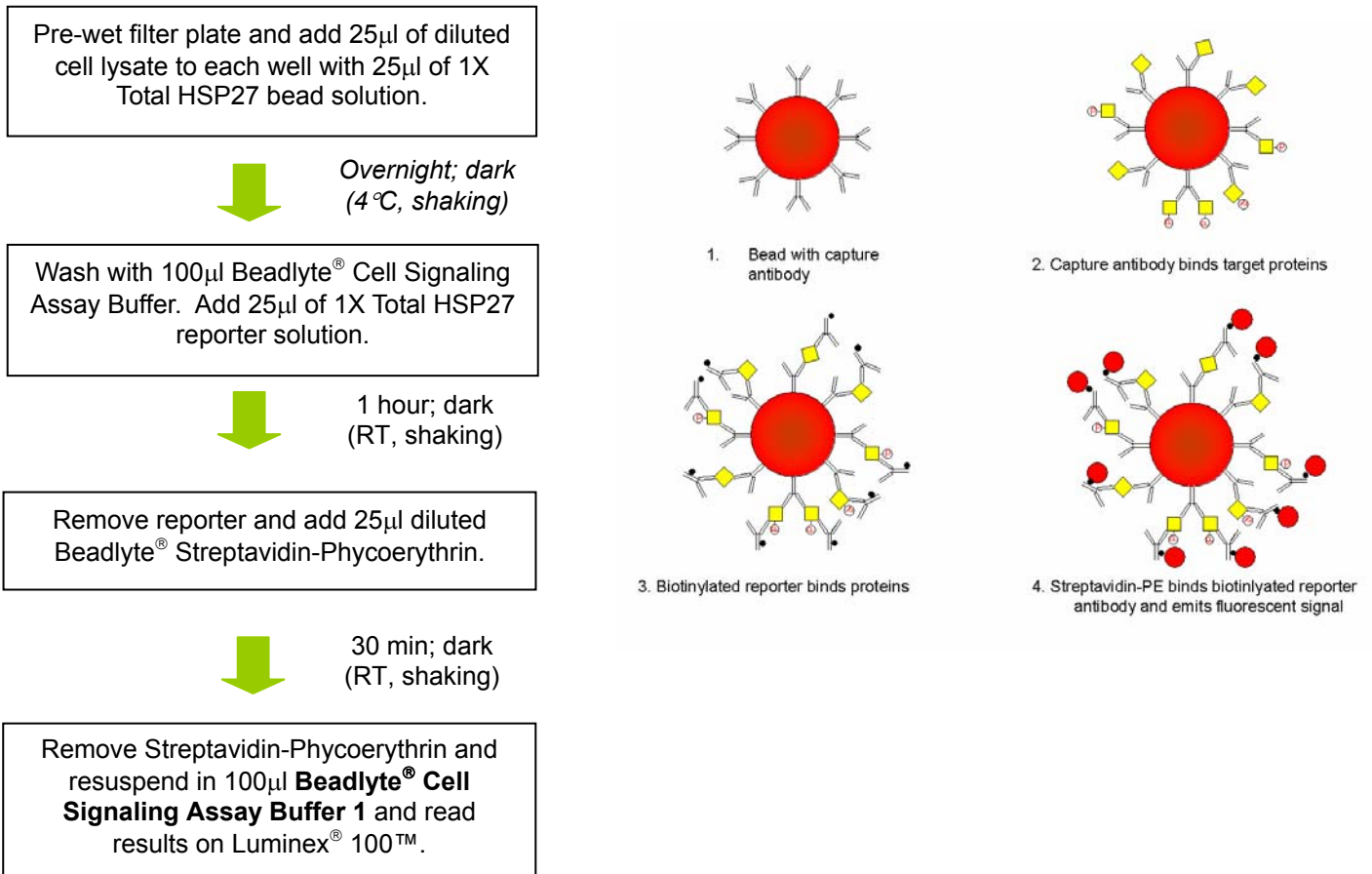
<http://www.upstate.com/img/coa/48-600-24907.pdf>

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[™] 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[®] 100[™] LabMAP[™] 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 HSP27 analysis. This lysate buffer is included in the Beadlyte[®] Cell Signaling Buffer Kit (Catalog # 48-600). Refer to the Beadlyte[®] 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[™], please refer to the Buffer Selection Table in the Beadlyte[®] 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[®] Cell Signaling **Assay Buffer 3** is recommended for best results (Beadlyte[®] Cell Signaling Buffer Kit, Catalog # 48-600).

Note: If Total HSP27 beads are being multiplexed with other Beadmates[™], please refer to the Buffer Selection Table in the Beadlyte[®] 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 [™]	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	100
			C	80-100	20-40	60-80

Representative Data:

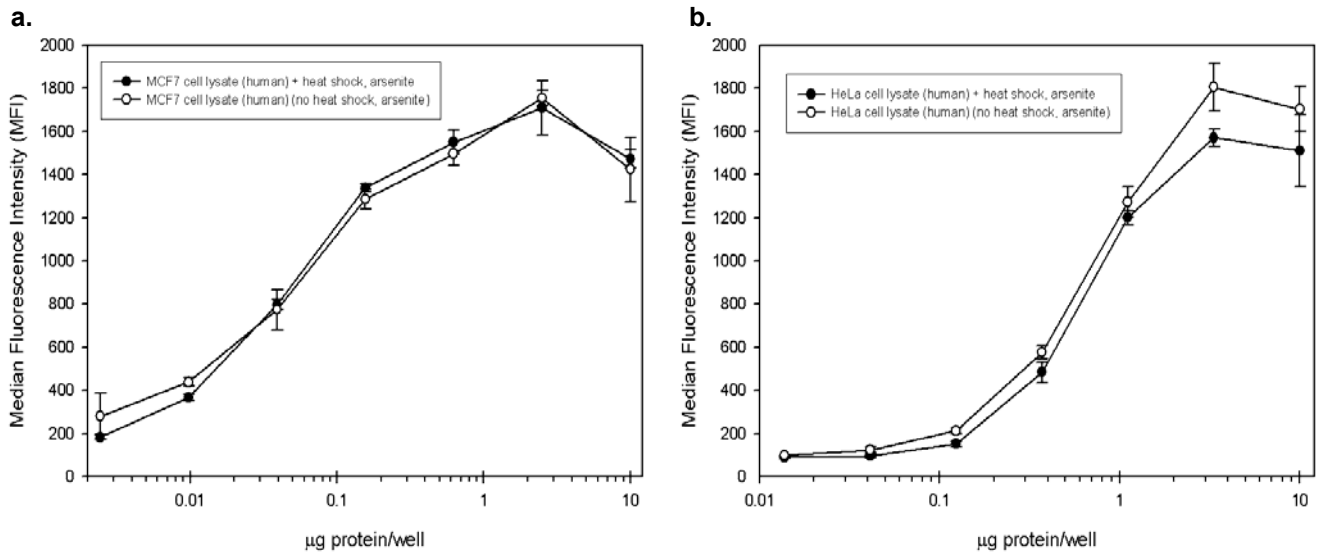
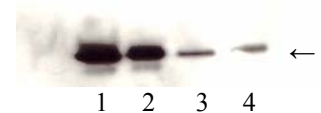


Figure 1. Beadlyte[®] detection of total HSP27 proteins in MCF7 cell lysate (a) and HeLa cell lysate (b). MCF7 cells (a) were grown to confluence and the cells to be stimulated (●) were heat shocked at 42°C for 30 minutes, while the unstimulated cells (○) were kept at 37°C. The cells were then grown 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[®] Lysis Buffer A with protease inhibitors) were incubated overnight at 4°C with Beadlyte[®] Anti-HSP27 Beads. The Beads were washed and mixed at room temperature with Beadlyte[®] Anti-Total HSP27, Biotin, followed by streptavidin-PE. The Median Fluorescence Intensity (MFI) was measured using the Luminex[®] 100™ 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[®] 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 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.