



cell signaling solutions

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## Certificate of Analysis

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### Active Caspase 3 Beadmates™

(100 Assay Points)

Catalog # 46-604

Lot # 27473

#### Components

**Beadlyte® Anti-Caspase 3 Beads**, Catalog # 42-604, Lot # 27473. One vial containing **125µl** of anti-Caspase 3 rabbit IgG conjugated to Luminex® Bead #6 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-Active Caspase 3, Biotin**, Catalog # 44-604, Lot # 27473. One vial containing **125µl** of biotin conjugated anti-Active Caspase 3 rabbit IgG (**20X**) in a proprietary formulation of Tris buffered salts and animal protein containing 0.05% sodium azide as a preservative.

**Specificity:** The assay recognizes epitopes on active caspase 3 which become accessible upon cleavage of the inactive proform.

**Applications:** Optimal antibody pair for detection of Active Caspase 3. 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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#### Active Caspase 3 Beadmate™ Description

**Use:** The Active Caspase 3 Beadmate™ pair is used in conjunction with the Beadlyte® Cell Signaling Buffer Kit (Catalog # 48-600) to detect the presence of activated caspase 3 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 for the Beadlyte® 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>®</sup> 100™ System
- Beadlyte<sup>®</sup> 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 with beads coupled to a caspase 3 specific capture antibody overnight. The beads are washed and mixed with a biotinylated active caspase 3 specific reporter, followed by streptavidin-phycoerythrin. The amount of active caspase 3 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 levels of activated caspase 3.

Pre-wet filter plate and add 25 $\mu$ l of diluted cell lysate to each well with 25 $\mu$ l of 1X Active Caspase 3 bead solution.



*Overnight; dark  
(4 °C, shaking)*

Wash with 100 $\mu$ l Beadlyte<sup>®</sup> Cell Signaling Assay Buffer and add 25 $\mu$ l of 1X Active Caspase 3 reporter solution.



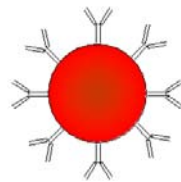
*1 hour; dark  
(RT, shaking)*

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

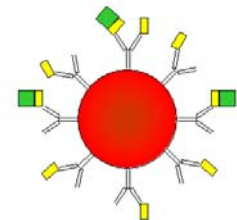


*30 min; dark  
(RT, shaking)*

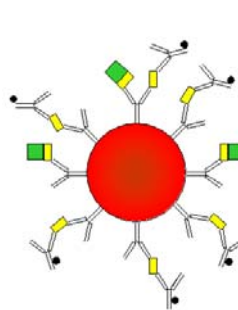
Remove Streptavidin-Phycoerythrin and resuspend in 100 $\mu$ l **Beadlyte<sup>®</sup> Cell Signaling Assay Buffer 1** and read results on Luminex<sup>®</sup> 100™.



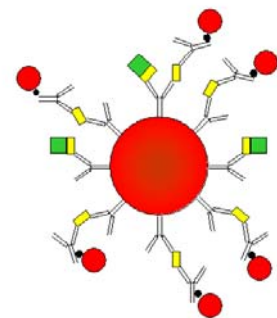
1. Bead with capture antibody



2. Capture antibody binds Caspase 3



3. Biotinylated reporter binds the active caspase 3 subunit



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

## Recommendations for Protocol

### Preparation of lysates

For a single plex analysis, Beadlyte<sup>®</sup> Cell Signaling **Lysis Buffer A** is recommended for lysing cells for Active Caspase 3 single plex 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 used in a multiplex assay with Active Caspase 3 and other Beadmates™, 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 Active Caspase 3 Beads and reporter antibodies

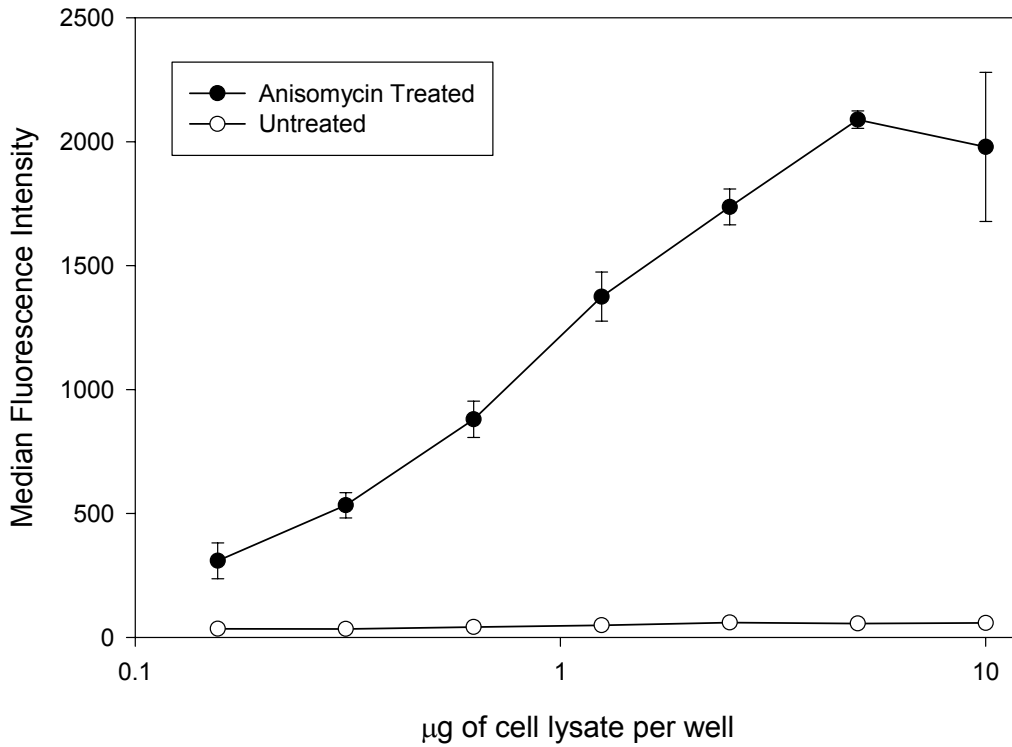
For Active Caspase 3 single plex analysis, Beadlyte<sup>®</sup> Cell Signaling **Assay Buffer 1** is recommended for best results (Beadlyte<sup>®</sup> Cell Signaling Buffer Kit, Catalog # 48-600).

**Note:** If Active Caspase 3 beads are being multiplexed with other Beadmates™, 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.

## Active Caspase 3 Buffer Selection Chart

Beadmate	Catalog #	Bead #	Lysis buffer	Assay buffer 1 activity (%)	Assay buffer 2 activity (%)	Assay buffer 3 activity (%)
Active Caspase 3	46-604	#6	A	<b>100</b>	20-40	20-40
			B	0	0	0
			C	60-80	0-20	20-40

**Representative Data:**



**Figure 1. Beadlyte<sup>®</sup> detection of active Caspase 3 in Jurkat cell lysates.** Human Jurkat T cells were treated with 20µM Anisomycin or medium for four hours to induce apoptosis (Catalog # 17-355). T cells (treated and untreated) were lysed using Beadlyte<sup>®</sup> Cell Signaling Lysis Buffer A containing protease inhibitors. Increasing amounts of cell lysate were incubated overnight at 4°C with Beadlyte<sup>®</sup> anti-Caspase 3 beads. The Beads were washed and mixed at room temperature with Beadlyte<sup>®</sup> anti-active Caspase 3, Biotin, followed by Beadlyte<sup>®</sup> Streptavidin-Phycoerythrin. The Median Fluorescence Intensity (MFI) of each well was measured using the Luminex<sup>®</sup> 100™ system. The average MFI and standard deviation from three wells were used to create the graph above.

**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<sup>®</sup> 100™. One or more of the following US patents covers this product and the use thereof: #6,046,807, #5,981,180.