



cell signaling solutions

## Certificate of Analysis

10 Old Barn Road • Lake Placid, NY 12946  
Technical Support: T: 800 548-7853 • F: 518 523-4513  
email: techserv@upstate.com  
Sales Department: T: 800 233-3991 • F: 781 890-7738  
Licensing Dept.: 800 310-4659  
www.upstate.com

### Beadlyte<sup>®</sup> Total Akt/PKB Beadmates™

(100 Assay Points)

Catalog # 46-605

Lot # 22350

#### Components

**Beadlyte<sup>®</sup> Total Anti-Akt/PKB Capture Beads**, Catalog # 42-605, Lot # 22350. One vial containing **125µl** of anti-Akt/PKB mouse IgG conjugated to Luminex™ Bead #38 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<sup>®</sup> Biotinylated Anti-Akt/PKB Reporter**, Catalog # 44-605, Lot # 22350. One vial containing **125µl** of anti-Akt/PKB 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 and mouse Akt/PKB, both phosphorylated and non-phosphorylated forms.

**Applications:** Optimal antibody pair for detection of Total Akt/PKB. 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 Akt/PKB Beadmate™ Description

**Use:** The Beadlyte<sup>®</sup> Total Akt/PKB Beadmate™ pair is used in conjunction with the Beadlyte<sup>®</sup> Cell Signaling Buffer Kit (Catalog # 48-600) to detect the presence of total Akt/PKB in cell lysates using the Luminex<sup>100</sup> system. Each Beadmate™ 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™. 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-Akt/PKB Beadmate™ pair CANNOT be multiplexed with the Beadlyte<sup>®</sup> Phospho-Akt/PKB Beadmate™ pair (Catalog # 46-601) 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 Akt/PKB at a site distinct from the secondary reporter site, capturing both phosphorylated and non-phosphorylated forms of Akt/PKB. The beads are washed and mixed with a biotinylated total Akt/PKB specific reporter, followed by streptavidin-phycoerythrin. The amount of total Akt/PKB is then quantified using the Luminex<sup>100</sup> LabMAP™ System.

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



Overnight; dark  
(4 °C, shaking)

Wash with 100 $\mu$ l Beadlyte® Cell Signaling Assay Buffer. Add 25 $\mu$ l of 1X Total Akt/PKB total reporter solution.



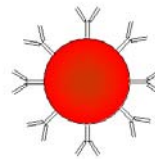
1 hour; dark  
(RT, shaking)

Remove reporter and add 25 $\mu$ l diluted Beadlyte™ Streptavidin-Phycoerythrin.

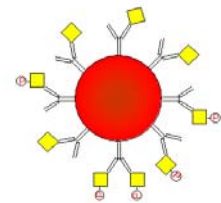


30 min; dark  
(RT, shaking)

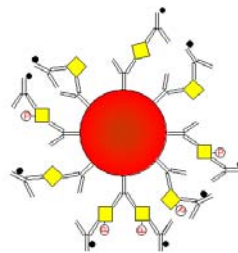
Remove Streptavidin-Phycoerythrin and resuspend in 100 $\mu$ l **Beadlyte® Cell Signaling Assay Buffer 1** and read results on Luminex<sup>100</sup>.



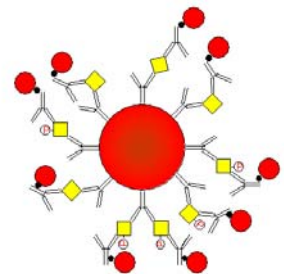
1. Bead with capture antibody



2. Capture antibody binds target proteins



3. Biotinylated reporter binds proteins



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 Total Akt/PKB 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 Akt/PKB 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 Akt/PKB Beads and reporter antibodies

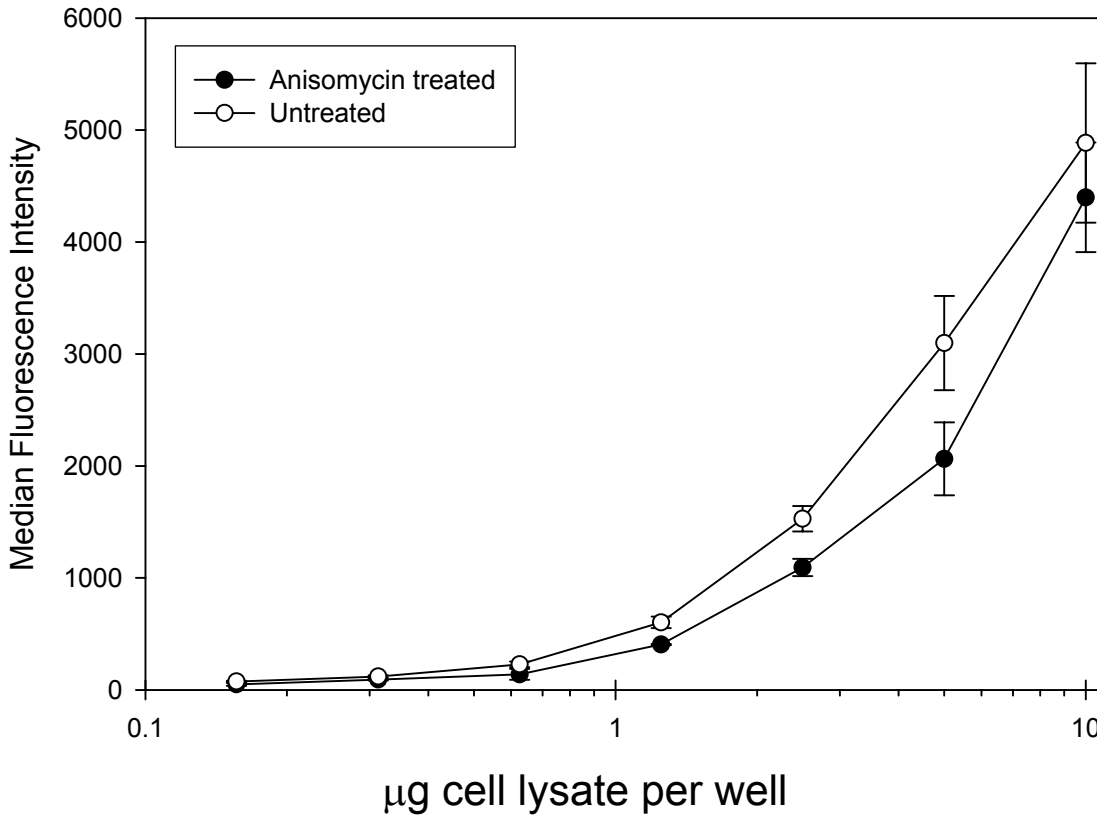
For Total Akt/PKB single plex analysis, Beadlyte<sup>®</sup> Cell Signaling **Assay Buffer 3** is recommended for best results (Beadlyte<sup>®</sup> Cell Signaling Buffer Kit, Catalog # 48-600).

**Note:** If Total Akt/PKB 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 Akt/PKB Buffer Selection Chart

Beadmate <sup>™</sup>	Catalog #	Bead #	Lysis buffer	Assay buffer 1 activity (%)	Assay buffer 2 activity (%)	Assay buffer 3 activity (%)
Total Akt/PKB	46-605	#38	A	80-100	20-40	<b>100</b>
			B	0-20	0	0
			C	0-20	0	0-20

**Representative Data:**



**Figure 1. Beadlyte<sup>®</sup> detection of Total Akt/PKB proteins in Jurkat T cell lysates.** Human Jurkat T cells were grown to confluence and stimulated with (●) or without (○) 20µM anisomycin for 30 minutes. Increasing amounts of cell lysate (lysed in Beadlyte<sup>®</sup> Cell Signaling Lysis Buffer A protease inhibitors) were incubated overnight at 4°C with Beadlyte<sup>®</sup> Total Anti-Akt/PKB Capture Beads. The Beads were washed and mixed at room temperature with Beadlyte<sup>®</sup> Biotinlyated Total Anti-Akt/PKB Reporter, followed by streptavidin-PE. The Median Fluorescence Intensity (MFI) was measured using the Luminex<sup>100</sup> LabMAP system.

**End-User License Agreement**

By purchasing this product, which contains fluorescently labeled microsphere beads authorized by Luminex<sup>™</sup>, you, the customer, acquire the right under Luminex's<sup>™</sup> 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<sup>™</sup> 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