

---

## Certificate of Analysis

**Total Akt/PKB Beadmates™**  
(100 Assay Points)  
Catalog # 46-605  
Lot # 0611044665

### Components

**Beadlyte® Anti-Akt/PKB Beads**, Catalog # 42-605, Lot # 0611044665. One vial containing **125µl** of anti-Akt/PKB mouse IgG conjugated to Luminex® Bead #38 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-Akt/PKB, Biotin**, Catalog # 44-605, Lot # 0611044665. 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® 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 Akt/PKB Beadmate™ Description

**Use:** The Total Akt/PKB Beadmate™ pair is used in conjunction with the Beadlyte® Cell Signaling Buffer Kit (Catalog # 48-600) to detect the presence of total Akt/PKB 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 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 (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 $\mu$ 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 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>®</sup> 100™ 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<sup>®</sup> Cell Signaling Assay Buffer. Add 25 $\mu$ l of Total Akt/PKB total reporter solution.



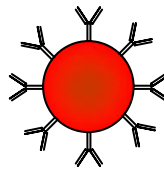
1 hour; dark  
(RT, shaking)

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

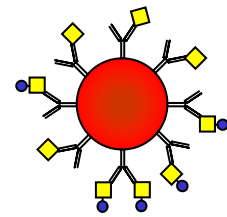


30 min; dark  
(RT, shaking)

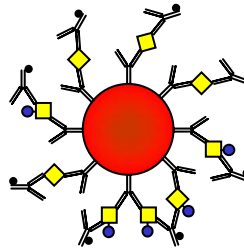
Remove Beadlyte<sup>®</sup> Streptavidin-Phycoerythrin and resuspend in 100 $\mu$ l Beadlyte<sup>®</sup> Cell Signaling Assay Buffer 1 and read results on Luminex<sup>®</sup> 100™.



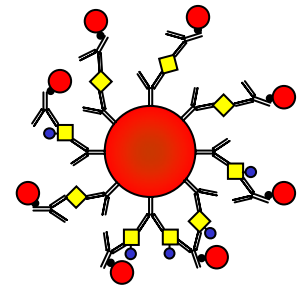
1. Bead with capture antibody



2. Capture antibody binds target proteins



3. Biotinylated reporter binds proteins



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

## Preparations for Assay Protocol

### Single-plex analysis

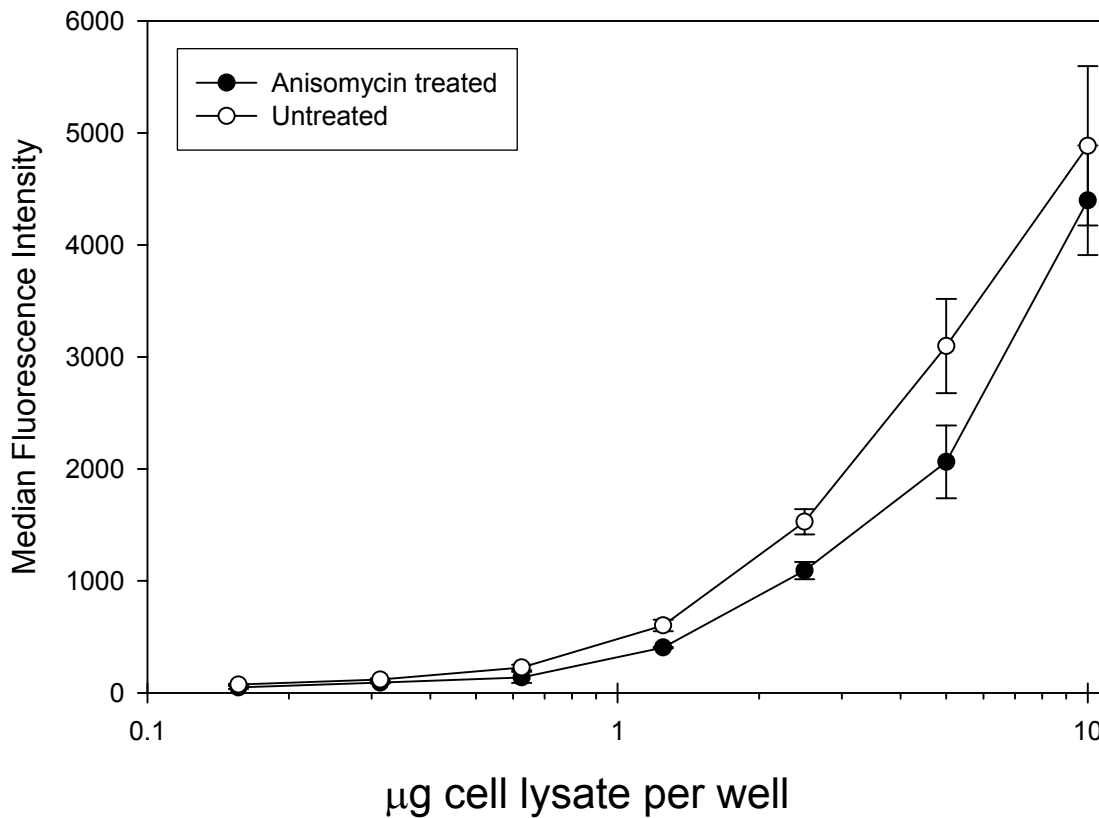
The recommended lysis and assay buffers for a single-plex analysis of Total Akt1/PKB $\alpha$  are Beadlyte<sup>®</sup> Cell Signaling Lysis Buffer A (Catalog # 43-018) and Beadlyte<sup>®</sup> Cell Signaling Assay Buffer 3 (Catalog # 43-012). Both buffers are included in the Beadlyte<sup>®</sup> Cell Signaling Buffer Kit (Catalog # 48-600). For the cell signaling assay and cell lysis protocols refer to the Beadlyte<sup>®</sup> Cell Signaling Buffer Kit COA (select the highest lot number) at: <http://www.upstate.com/browse/productdetail.asp?ProductId=48-600>.

### Multiplex analysis

The use of Beadlyte<sup>®</sup> Cell Signaling Universal Buffers for multiplexing Total Akt1/PKB $\alpha$  Beadmates<sup>™</sup> is not recommended at this time. For multiplexing Total Akt1/PKB $\alpha$  Beadmates<sup>™</sup> with other Beadmates<sup>™</sup> select the optimal lysis and assay buffers using the Buffer Selection Table in the Beadlyte<sup>®</sup> Cell Signaling Buffer Kit COA (Catalog # 48-600). The cell signaling assay and cell lysis protocols are also provided in the Beadlyte<sup>®</sup> 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<sup>™</sup> should not be multiplexed together.

**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> Anti-Akt/PKB Beads. The Beads were washed and mixed at room temperature with biotinylated Beadlyte<sup>®</sup> Anti-Akt/PKB, followed by Beadlyte<sup>®</sup> Streptavidin-Phycoerythrin. The Median Fluorescence Intensity (MFI) was measured using the Luminex<sup>®</sup> 100™ System.

**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.