



cell signaling solutions

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*Certificate of Analysis*

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**Beadlyte<sup>®</sup> Phospho-Akt/PKB(Ser 473) Beadmates<sup>™</sup>**

(100 Assay Points)

Catalog # 46-601

Lot # 22299

**Components**

**Beadlyte<sup>®</sup> Anti-Akt/PKB Capture Beads**, Catalog # 42-601, Lot # 22299. One vial containing **125 $\mu$ l** of anti-Akt/PKB mouse monoclonal IgG conjugated to Luminex<sup>™</sup> Bead #38 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 Anti-phospho-Akt/PKB Reporter**, Catalog # 44-601, Lot #22299. One vial containing **125 $\mu$ l** of anti-phospho-Akt/PKB(Ser 473) 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 phosphorylated on Ser473.

**Applications:** Optimal antibody pair for detection of Akt/PKB phosphorylated on Ser473. 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 IN VITRO 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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**Phospho-Akt/PKB Beadmate<sup>™</sup> Description**

**Use:** The Beadlyte<sup>®</sup> Phospho-Akt/PKB 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 phosphorylated Akt/PKB (Ser 473) in cell lysates using the Luminex<sup>100</sup> LabMAP<sup>™</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> Phospho-Akt/PKB Beadmate<sup>™</sup> pair CANNOT be multiplexed with the Beadlyte<sup>®</sup> Total Akt/PKB Beadmate<sup>™</sup> pair (Catalog # 46-605) 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)

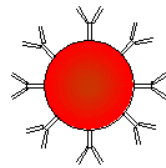
### Detection Protocol Summary

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 with beads coupled to an Akt/PKB specific capture antibody overnight. The beads are washed and mixed with a biotinylated phospho-Akt/PKB specific reporter, followed by streptavidin-phycoerythrin. The amount of phospho-Akt/PKB is then quantified using the Luminex<sup>100</sup> LabMAP™ System. A sample with unstimulated cell lysate and containing all other components will give the value for any basal phosphorylation of Akt/PKB.

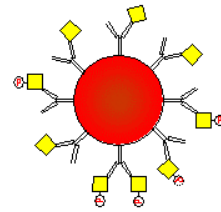
Pre-wet filter plate and add 25 $\mu$ l of diluted cell lysate to each well with 25 $\mu$ l of 1X Akt/PKB bead solution.



*Overnight; dark  
(4 °C, shaking)*



1. Bead with capture antibody

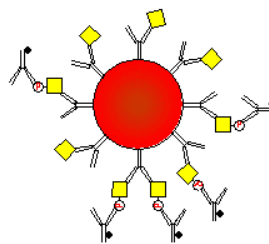


2. Capture antibody binds target proteins

Wash with 100 $\mu$ l Beadlyte® Cell Signaling Assay Buffer and add 25 $\mu$ l of 1X Phospho-Akt/PKB reporter solution.



*1 hour; dark  
(RT, shaking)*

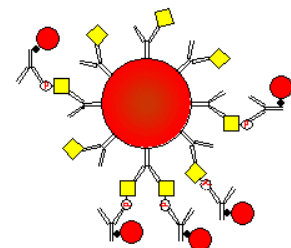


3. Biotinylated reporter binds phosphorylated proteins

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



*30 min; dark  
(RT, shaking)*



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

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

## 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 Phospho Akt/PKB 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 Phospho-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 Phospho-Akt/PKB Beads and reporter antibodies

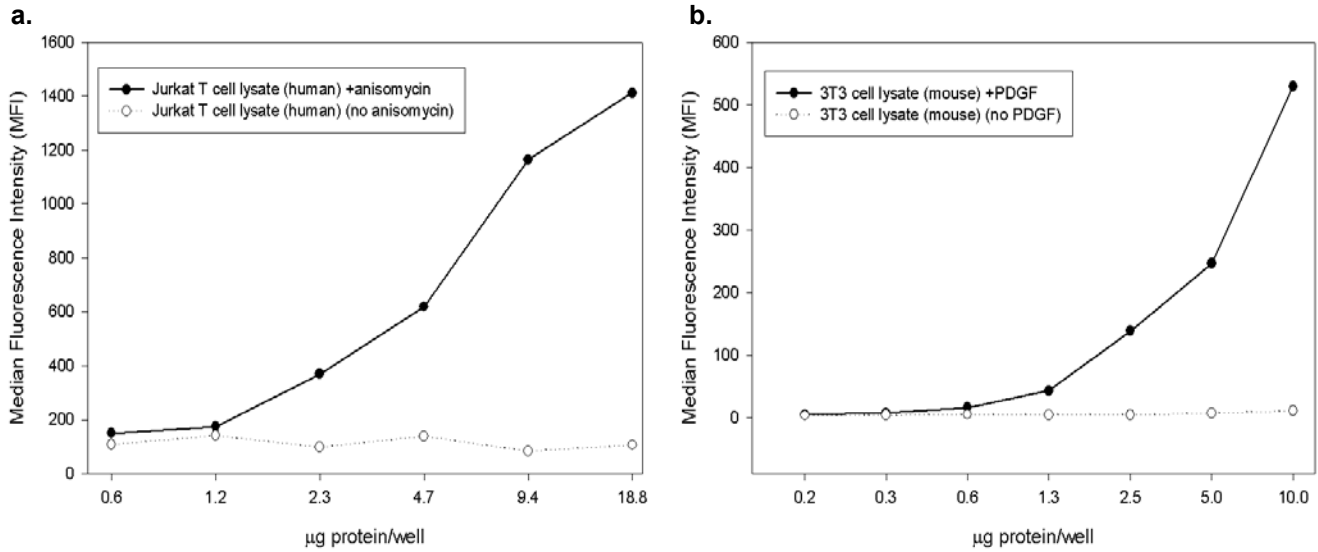
For Phospho-Akt/PKB single plex analysis, Beadlyte<sup>®</sup> Cell Signaling **Assay Buffer 1** is recommended for best results (Beadlyte<sup>®</sup> Cell Signaling Buffer Kit, Catalog # 48-600).

**Note:** If Phospho-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.

## Phospho-Akt/PKB Buffer Selection Chart

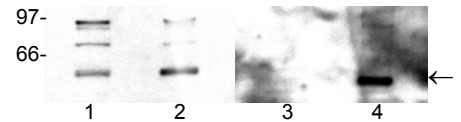
Beadmate	Catalog #	Bead #	Lysis buffer	Assay buffer 1 activity (%)	Assay buffer 2 activity (%)	Assay buffer 3 activity (%)
Phospho-Akt/PKB	46-601	#38	A	<b>100</b>	20-40	40-60
			B	0-20	0	0
			C	20-40	0	0-20

**Representative Data:**



**Figure 1. Beadlyte<sup>®</sup> detection of phosphorylated Akt/PKB proteins in Jurkat T cell lysate (a) and 3T3 (b) cell lysate.** Jurkat T cells (a) were grown to confluence and stimulated with (●) or without (○) 20µM anisomycin for 30 minutes. 3T3 cells (b) were grown to confluence and stimulated with (●) or without (○) 50ng/ml PDGF for 15 minutes. Increasing amounts of cell lysate (lysed in Beadlyte<sup>®</sup> Cell Signaling Lysis Buffer A with protease inhibitors) were incubated overnight at 4°C with Beadlyte<sup>®</sup> Anti-Akt/PKB Capture Bead. The Beads were washed and mixed at room temperature with Beadlyte<sup>®</sup> Biotinylated Anti-phospho-Akt/PKB Reporter, followed by streptavidin-PE. The Median Fluorescence Intensity (MFI) was measured using the Luminex<sup>100</sup> LabMAP<sup>™</sup> system.

**Figure 2. Western blot detection of phosphorylated Akt/PKB in Jurkat T cell lysate and 3T3 cell lysate.** Jurkat T cells were grown to confluence and stimulated with or without 20µM anisomycin for 30 minutes. 3T3 cells were grown to confluence and stimulated with or without 50ng/ml PDGF for 15 minutes. 10µg/well of unstimulated Jurkat (lane 1), stimulated Jurkat (lane 2), unstimulated 3T3 (lane 3) or stimulated 3T3 (lane 4) cell lysate (lysed in Beadlyte<sup>®</sup> Cell Signaling Lysis Buffer A with protease inhibitors) were separated by SDS-PAGE, transferred to nitrocellulose, and probed with rabbit anti-phospho-Akt/PKB. Blots were incubated with HRP labeled anti-rabbit IgG and visualized via chemiluminescence. Arrow indicates phosphorylated Akt/PKB (60 kDa).



**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.