



cell signaling solutions

Certificate of Analysis

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Beadlyte[®] Phospho-Akt/PKB (Ser473) Beadmates[™]

(100 Assay Points)

Catalog # 46-601

Lot # 24843

Components

Beadlyte[®] Phospho-Akt/PKB Beads, Catalog # 42-601, Lot # 24843. One vial containing **125 μ l** of anti-Akt/PKB mouse monoclonal IgG conjugated to Luminex[™] Bead #38 at **2000 beads/ μ l (20X)** in a proprietary formulation of Tris buffered salts and animal protein containing 0.05% sodium azide as a preservative.

Beadlyte[®] Phospho-Akt/PKB Reporter, Catalog # 44-601, Lot #24843. One vial containing **125 μ 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[®] 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**

Phospho-Akt/PKB Beadmate[™] Description

Use: The Beadlyte[®] Phospho-Akt/PKB Beadmate[™] pair is used in conjunction with the Beadlyte[®] Cell Signaling Buffer Kit (Catalog # 48-600) to detect the presence of phosphorylated Akt/PKB (Ser 473) in cell lysates using the Luminex¹⁰⁰ LabMAP[™] 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 Beadlyte[®] Phospho-Akt/PKB Beadmate[™] pair CANNOT be multiplexed with the Beadlyte[®] Total Akt/PKB Beadmate[™] 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[®] 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)
- Luminex¹⁰⁰ LabMAP™ System
- Beadlyte® Cell Signaling Buffer Kit (Catalog # 48-600)

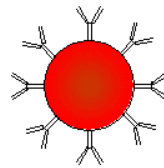
Detection Protocol Summary

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 Luminex¹⁰⁰ LabMAP™ System. A sample with unstimulated cell lysate and containing all other components will give the value for any basal phosphorylation of Akt/PKB.

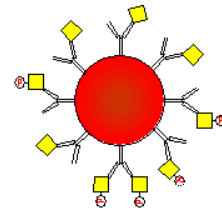
Pre-wet filter plate and add 25 μ l of diluted cell lysate to each well with 25 μ l of 1X Akt/PKB bead solution.



*Overnight; dark
(4 °C, shaking)*



1. Bead with capture antibody

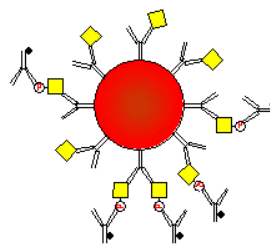


2. Capture antibody binds target proteins

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



*1 hour; dark
(RT, shaking)*

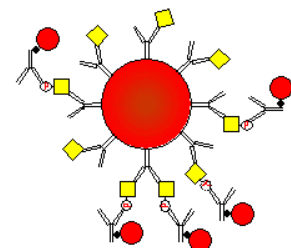


3. Biotinylated reporter binds phosphorylated proteins

Remove reporter and add 25 μ 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 μ l **Beadlyte® Cell Signaling Assay Buffer 1** and read results on Luminex¹⁰⁰.

Recommendations for Protocol

Preparation of lysates

For a single plex analysis, Beadlyte[®] 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[®] 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 used in a multiplex assay with Phospho-Akt/PKB 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 Phospho-Akt/PKB Beads and reporter antibodies

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

Note: If Phospho-Akt/PKB 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.

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	100	20-40	40-60
			B	0-20	0	0
			C	20-40	0	0-20

Representative Data:

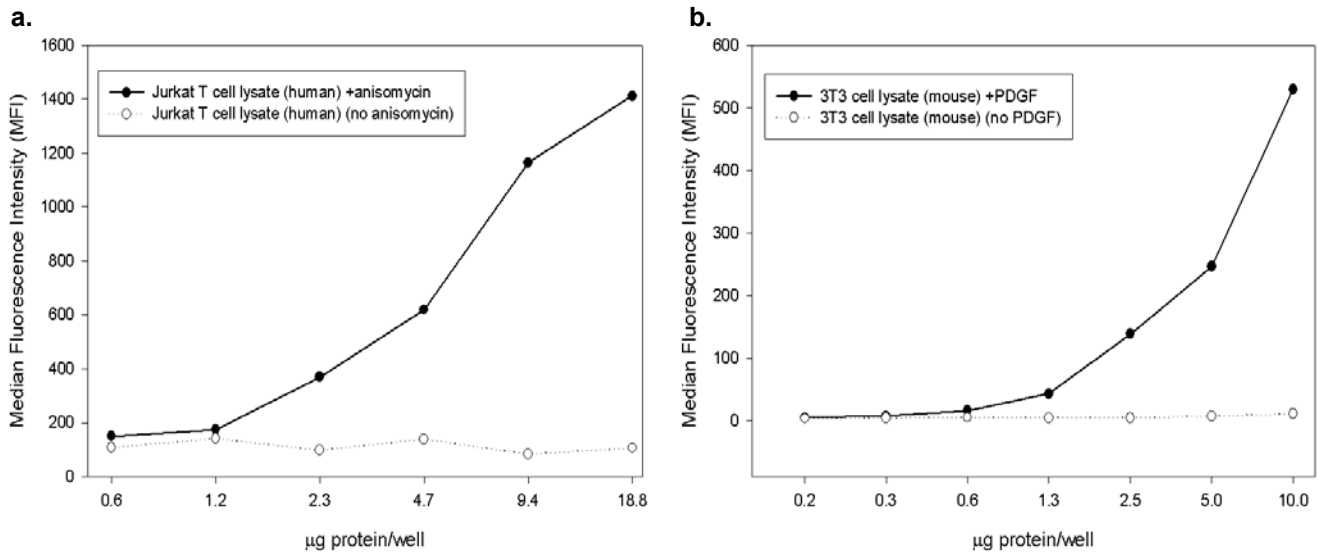
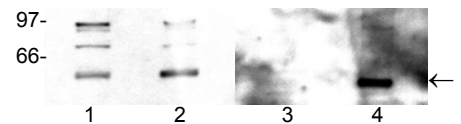


Figure 1. Beadlyte[®] detection of phosphorylated Akt/PKB proteins in Jurkat T cell lysate (a) and 3T3 (b) cell lysate. Jurkat T cells (a) were grown to confluence and stimulated with (●) or without (○) 20µM anisomycin for 30 minutes. 3T3 cells (b) were grown to confluence and stimulated with (●) or without (○) 50ng/ml PDGF for 15 minutes. Increasing amounts of cell lysate (lysed in Beadlyte[®] Cell Signaling Lysis Buffer A with protease inhibitors) were incubated overnight at 4°C with Beadlyte[®] Phospho-Akt/PKB Bead. The Beads were washed and mixed at room temperature with Beadlyte[®] Phospho-Akt/PKB Reporter, followed by Streptavidin-Phycoerythrin. The Median Fluorescence Intensity (MFI) was measured using the Luminex¹⁰⁰ LabMAP[™] 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[®] 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 (60kDa).



End-User License Agreement

By purchasing this product, which contains fluorescently labeled microsphere beads authorized by Luminex[™], you, the customer, acquire the right under Luminex'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 Luminex¹⁰⁰. This product and the use thereof are covered by one or more of the following US patents: # 6,046,807, # 5,981,180.