



cell signaling solutions

Certificate of Analysis

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Beadlyte[®] Total Akt/PKB Beadmates[™]
(100 Assay Points)
Catalog # 46-605
Lot # 24866

Components

Beadlyte[®] Total Akt/PKB Beads, Catalog # 42-605, Lot # 24866. One vial containing **125 μ l** of anti-Akt/PKB mouse 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[®] Akt/PKB Reporter, biotin, Catalog # 44-605, Lot # 24866. One vial containing **125 μ l** 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 Beadlyte[®] 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 Luminex¹⁰⁰ 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[®] Total-Akt/PKB Beadmate[™] pair CANNOT be multiplexed with the Beadlyte[®] 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[®] 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 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 Luminex¹⁰⁰ LabMAP™ System.

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)*

Wash with 100 μ l Beadlyte[®] Cell Signaling Assay Buffer. Add 25 μ l of 1X Total Akt/PKB total reporter solution.



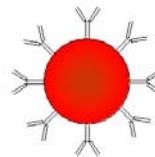
*1 hour; dark
(RT, shaking)*

Remove reporter and add 25 μ l diluted Beadlyte™ Streptavidin-Phycoerythrin.

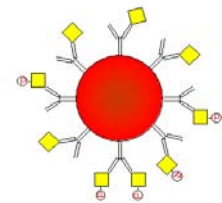


*30 min; dark
(RT, shaking)*

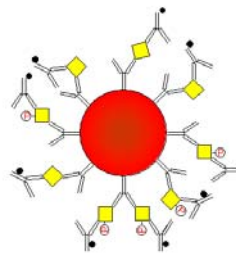
Remove Streptavidin-Phycoerythrin and resuspend in 100 μ l **Beadlyte[®] Cell Signaling Assay Buffer 1** and read results on Luminex¹⁰⁰.



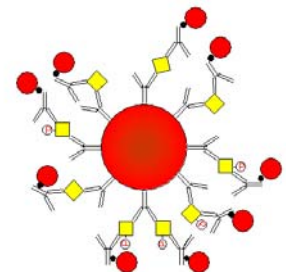
1. Bead with capture antibody



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[®] Cell Signaling **Lysis Buffer A** is recommended for lysing cells for Total Akt/PKB 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 multiplexed with Total 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 Total Akt/PKB Beads and reporter antibodies

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

Note: If Total 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.

Total Akt/PKB Buffer Selection Chart

Beadmate [™]	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	100
			B	0-20	0	0
			C	0-20	0	0-20

Representative Data:

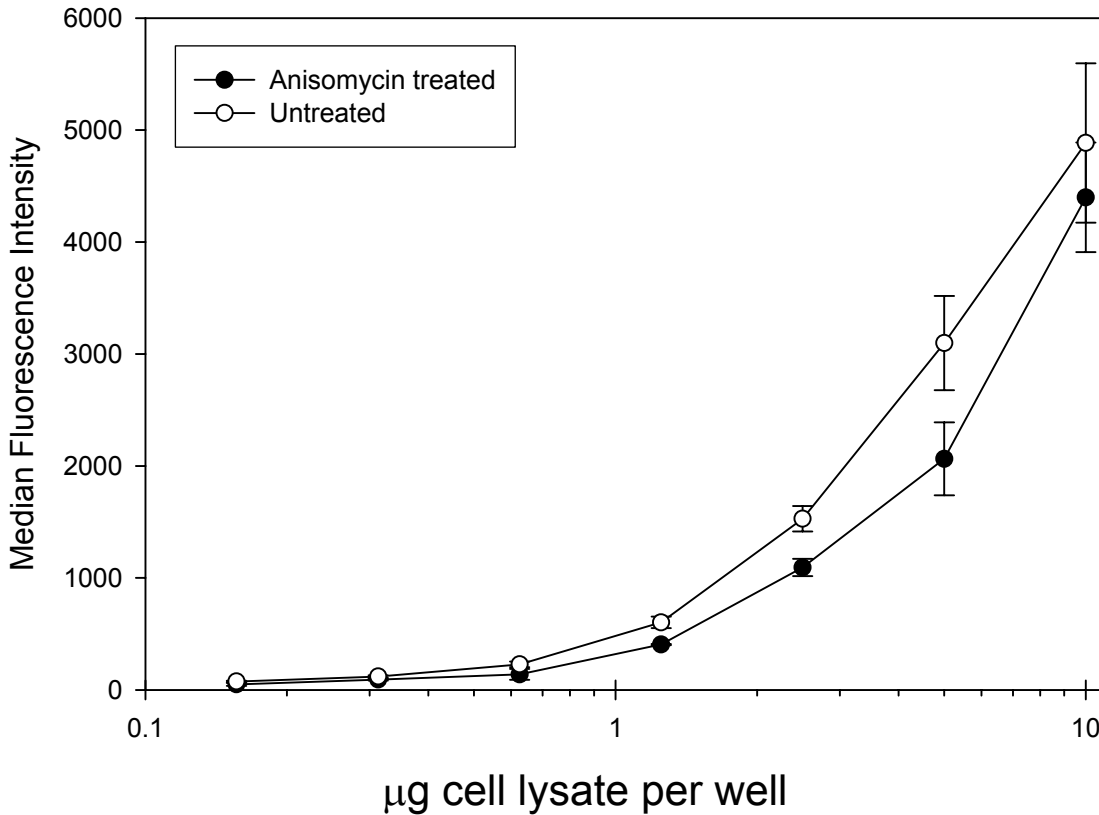


Figure 1. Beadlyte[®] detection of Total Akt/PKB proteins in Jurkat T cell lysates. Human Jurkat T cells were grown to confluence and stimulated with (●) or without (○) 20µM anisomycin for 30 minutes. Increasing amounts of cell lysate (lysed in Beadlyte[®] Cell Signaling Lysis Buffer A protease inhibitors)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

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