
Certificate of Analysis

Phospho-Akt1/PKB α (Ser473) Beadmates™

(100 Assay Points)

Catalog # 46-601

Lot # 0609040016

Components

Beadlyte® Anti-Akt1/PKB α Beads, Catalog # 42-601, Lot # 0609040016. One vial containing **125 μ l** of anti-Akt1/PKB α mouse monoclonal IgG conjugated to Luminex® Bead #38 at **4000 beads/ μ l (20X)** in a proprietary formulation of Tris buffered salts and animal protein containing 0.05% sodium azide as a preservative.

Beadlyte® Anti-phospho-Akt1/PKB α (Ser473), Biotin, Catalog # 44-601, Lot # 0609040016. One vial containing **125 μ l** of anti-phospho-Akt1/PKB α (Ser473) 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 Akt1/PKB α phosphorylated on Ser473.

Applications: Optimal antibody pair for detection of Akt1/PKB α 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-Akt1/PKB α (Ser473) Beadmate™ Description

Use: The Phospho-Akt1/PKB α (Ser473) Beadmate™ pair is used in conjunction with the Beadlyte® Cell Signaling Buffer Kit (Catalog # 48-600) to detect the presence of phosphorylated Akt1/PKB α (Ser473) 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 also ordered separately and consist 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 μ l) pipette + tips
- Sonication Bath (Catalog # 40-002)
- Millipore multiscreen vacuum manifold (Catalog # MAVM0960R)
- Luminex[®] 100™ System
- Beadlyte[®] Cell Signaling Buffer Kit (Catalog # 48-600)

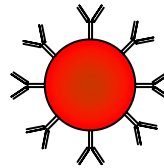
Detection Protocol Summary

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 Akt1/PKB α specific capture antibody overnight. The beads are washed and mixed with a biotinylated phospho-Akt1/PKB α specific reporter, followed by streptavidin-phycoerythrin. The amount of phospho-Akt1/PKB α is then quantified using the Luminex[®] 100™ System. A sample with unstimulated cell lysate and containing all other components will give the value for any basal phosphorylation of Akt1/PKB α .

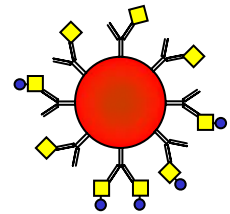
Pre-wet filter plate and add 25 μ l of diluted cell lysate to each well with 25 μ l of 1X Akt1/PKB α bead solution.



Overnight; dark
(4 °C, shaking)



1. Bead with capture antibody

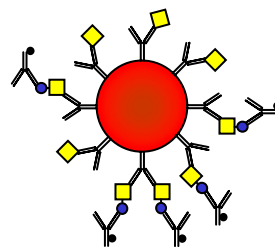


2. Capture antibody binds target proteins

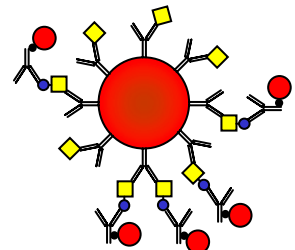
Wash with 100 μ l Beadlyte[®] Cell Signaling Assay Buffer and add 25 μ l of 1X anti-phospho-Akt1/PKB α (Ser473), biotin solution.



1 hour; dark
(RT, shaking)



3. Biotinylated reporter binds phosphorylated proteins



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

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 Luminex[®] 100™.

Preparations for Assay Protocol

Single-plex analysis

The recommended lysis and assay buffers for a single-plex analysis of Phospho-Akt1/PKB α (Ser473) are Beadlyte[®] Cell Signaling **Lysis Buffer A** (Catalog # 43-018) and Beadlyte[®] Cell Signaling **Assay Buffer 1** (Catalog # 43-010). Both buffers are included in the Beadlyte[®] Cell Signaling Buffer Kit (Catalog # 48-600). For the cell signaling assay and cell lysis protocols refer to the Beadlyte[®] 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[®] Cell Signaling Universal Buffers for multiplexing Phospho-Akt1/PKB α (Ser473) Beadmates[™] is not recommended at this time. For multiplexing Phospho-Akt1/PKB α (Ser473) Beadmates[™] with other Beadmates[™] select the optimal lysis and assay buffers using the Buffer Selection Table in the Beadlyte[®] Cell Signaling Buffer Kit COA (Catalog # 48-600). The cell signaling assay and cell lysis protocols are also provided in the Beadlyte[®] 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[™] should not be multiplexed together.

Representative Data:

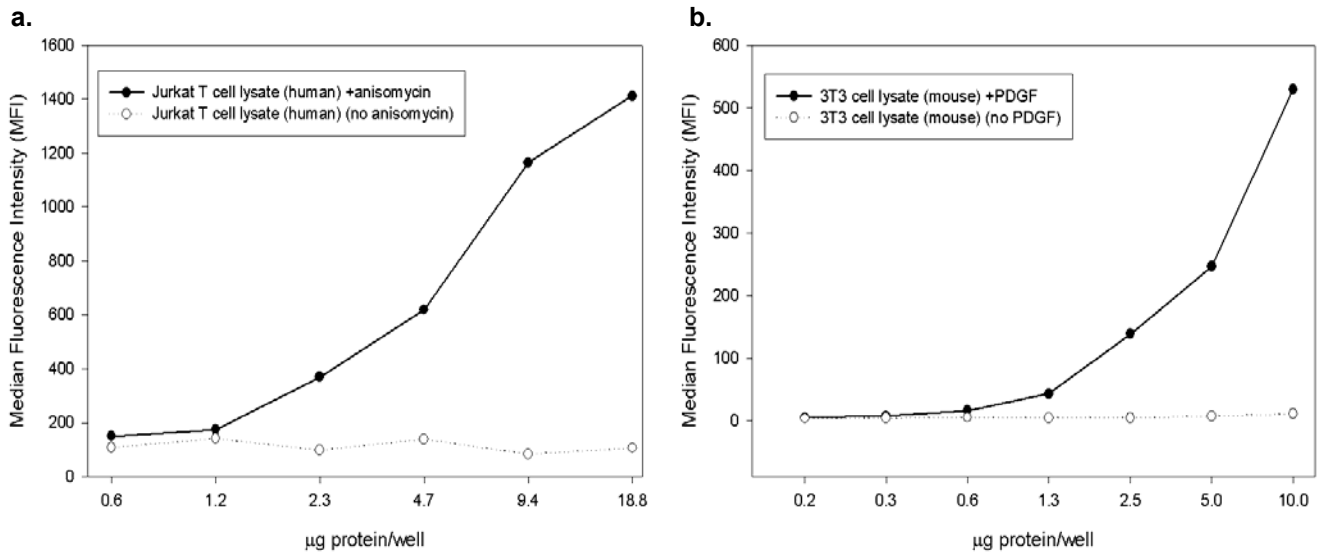
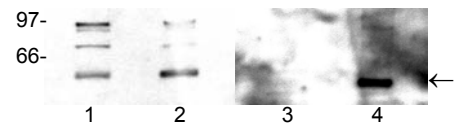


Figure 1. Beadlyte[®] detection of phosphorylated Akt1/PKB α proteins in Jurkat T cell lysate (a) and 3T3 (b) cell lysate. Jurkat T cells (a) were grown to confluence and stimulated with (●) or without (○) 20 μ M anisomycin for 30 minutes. 3T3 cells (b) were grown to confluence and stimulated with (●) 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[®] anti-Akt1/PKB α Beads. The Beads were washed and mixed at room temperature with Beadlyte[®] anti-phospho-Akt1/PKB α (Ser473), Biotin, followed by Streptavidin-Phycoerythrin. The Median Fluorescence Intensity (MFI) was measured using the Luminex[®] 100™ system.

Figure 2. Western blot detection of phosphorylated Akt1/PKB α in Jurkat T cell lysate and 3T3 cell lysate. Jurkat T cells were grown to confluence and stimulated with or without 20 μ M anisomycin for 30 minutes. 3T3 cells were grown to confluence and stimulated with or without 50ng/ml PDGF for 15 minutes. 10 μ g/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-Akt1/PKB α . Blots were incubated with HRP labeled anti-rabbit IgG and visualized via chemiluminescence. Arrow indicates phosphorylated Akt1/PKB α (60kDa).



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[®] 100™. One or more of the following US patents covers this product and the use thereof: #6,046,807, #5,981,180.