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Certificate of Analysis

Phospho-Akt1/PKBα (Thr308) Beadmates™

(100 Assay Points)
Catalog # 46-645
Lot #

Components

Beadlyte® Anti-Akt1/PKBα Beads, Catalog # 42-645, Lot #. One vial containing **131µl** of anti-Akt1/PKBα mouse IgG conjugated to Luminex® Bead #38 at **2400 beads/µl (20X)** in a proprietary formulation of Tris buffered salts and animal protein containing 0.05% sodium azide as a preservative.

Beadlyte® Anti-phospho-Akt1/PKBα (Thr308), Biotin, Catalog # 44-645, Lot #. One vial containing **131µl** of biotin conjugated anti-phospho-Akt1/PKBα (Thr308) 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/PKBα phosphorylated on Thr308.

Applications: Optimal antibody pair for detection of Akt1/PKBα phosphorylation on Thr308. To be used in conjunction with the Beadlyte® Cell Signaling Buffer Kits (Catalog #s 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

Phospho-Akt1/PKBα (Thr308) Beadmate™ Description

Use: The Phospho-Akt1/PKBα (Thr308) Beadmate™ pair is used in conjunction with Beadlyte® Cell Signaling Buffer Kits (Catalog # 48-600) to detect the presence of phospho-Akt1/PKBα (Thr308) 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 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 (5-200µl) 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/PKBα specific capture antibody overnight. The beads are washed and mixed with a biotinylated phospho-Akt1/PKBα specific reporter, followed by streptavidin-phycoerythrin. The amount of phospho-Akt1/PKBα 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 levels of phospho- Akt1/PKBα.

Pre-wet filter plate and add 25µl of diluted cell lysate to each well with 25µl of 1X Akt1/PKBα bead solution.

Overnight; dark (4°C, shaking)

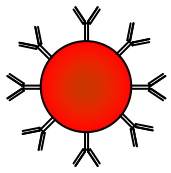
Wash with 100µl Beadlyte® Cell Signaling Assay Buffer and add 25µl of 1X anti-phospho-Akt1/PKBα (Thr308), biotin solution.

1 hour; dark (RT, shaking)

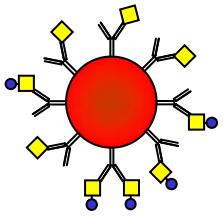
Remove reporter and add 25µl diluted Beadlyte® Streptavidin-Phycoerythrin.

30 min; dark (RT, shaking)

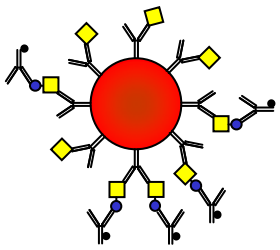
Remove Streptavidin-Phycoerythrin and resuspend in 100µl **Beadlyte® Cell Signaling Assay Buffer 1** and read results on Luminex® Instrument.



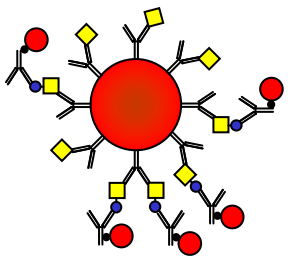
1. Bead with capture antibody



2. Capture antibody binds target proteins



3. Biotinylated reporter binds phosphorylated 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 Phospho-Akt1/PKBα (Thr308) Beadmates™ 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/PKBα (Thr308) Beadmates™ is not recommended at this time. For multiplexing Phospho-Akt1/PKBα (Thr308) 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.

Important Note: The Phospho-Akt1/PKBα (Thr308) Beadmates™ pair *CANNOT* be multiplexed with the Phospho-Akt1/PKBα (Ser473) (Catalog # 46-601).

Representative Data:

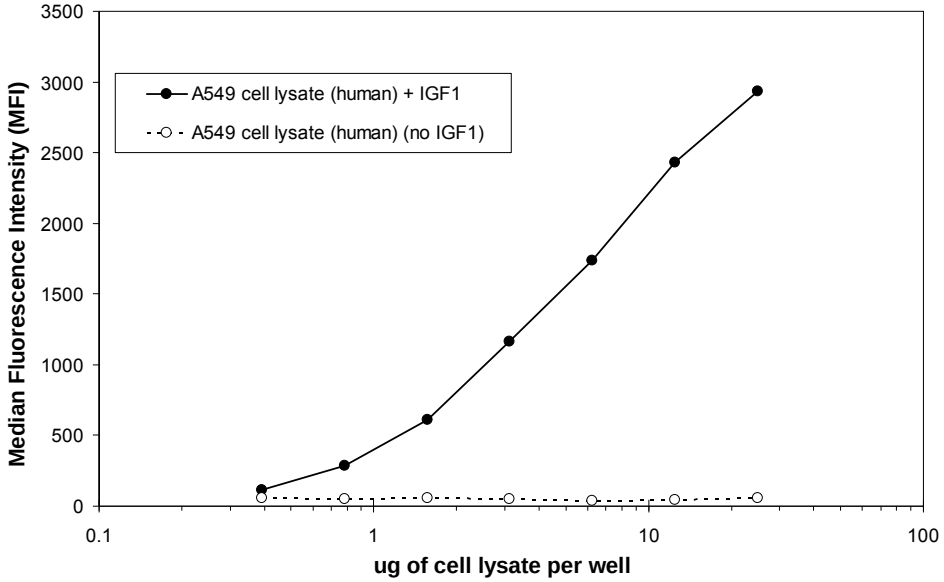


Figure 1. **Beadlyte® detection of phosphorylated Akt1/PKBα (Thr308) proteins in A549 cell lysate.** A549 cells were grown to confluence and stimulated with (●) or without (○) 50ng IGF1 for 15 minutes. Increasing amount of cell lysate (lysed in **Beadlyte® Cell Signaling Lysis Buffer A** with protease inhibitors) were incubated overnight at 4°C with **Beadlyte® anti-Akt1/PKBα beads**. The beads were washed and mixed at room temperature with **Beadlyte® anti-phospho-Akt1/PKBα (Ser308), Biotin**, followed by Streptavidin-PE. The Median Fluorescent Intensity (MFI) was measured using the Luminex® 100™ system. Figure 1 shows changes in phosphorylated Akt1/PKBa (Thr308) as detected with phospho-Akt1/PKBa (Thr308) Beadmates™.

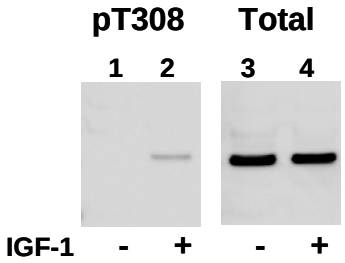


Figure 2. **Immunoprecipitation/Western Blot detection of phosphorylated Akt1/PKBα in A549 cell lysate.** 10μg of unstimulated (lanes 1 and 3) and IGF1 treated (lanes 2 and 4) A549 lysate (described in Figure 1) were mixed with capture antibody to immunoprecipitate Akt1/PKBα. The immunoprecipitated proteins were separated by SDS-PAGE, transferred to PVDF membrane, and probed with rabbit anti-phospho-Akt1/PKBα or anti-total Akt1/PKBα antibodies. Blots were incubated with HRP labeled anti-IgG antibody and visualized via chemiluminescence.