

Certificate of Analysis

Anti-phospho-Akt1/PKB α (Thr308), clone NL50

(rabbit monoclonal IgG)

Catalog # 05-802

Lot # JBC1350273

Immunogen: KLH-conjugated, synthetic peptide containing the sequence ..MK[pT]FC.. in which pT corresponds to phospho-threonine at residue 308 of mouse Akt. The Akt1 sequence is identical to human, rat, bovine and chicken Akt1. Human and mouse Akt2 shares 11/12 amino acids and rat Akt3 shares 10/12 amino acids with the peptide immunogen sequence.

Specificity: Recognizes phosphorylated Akt1/PKB α , Mr 60kDa. Cross-reactivity observed with Akt2, but **not** with Akt3.

Species Cross-reactivity: Human and mouse. Predicted to cross-react with rat, bovine and chicken based on sequence homology.

Formulation: 100 μ l of protein A purified rabbit monoclonal IgG in 0.2M Tris-glycine, pH 7.2, 0.15M NaCl and 0.05% sodium azide before the addition of glycerol to 30%. Liquid at -20°C.

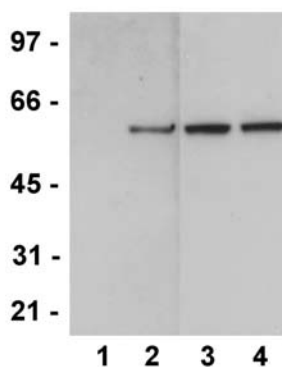
Storage and Stability: Stable for 2 years at -20°C from date of shipment. Aliquot to avoid repeated freezing and thawing. For maximum recovery of product, centrifuge the vial after thawing and prior to removing the cap.

**FOR RESEARCH USE ONLY
NOT FOR USE IN HUMANS**

Quality Control Testing

Immunoblot Analysis: A 1:1,000-1:5,000 dilution of this lot detected phospho-Akt1/PKB α in RIPA lysates from 3T3/NIH cells stimulated with 100ng/ml PDGF for 20 minutes (Figure A).

Beadlyte[®] Specificity Assay: 1:1,000-1:10,000 dilutions of this lot were incubated with phosphorylated and unphosphorylated recombinant Akt proteins conjugated to Luminex microspheres. Cross-reactivity to active Akt2 was detected (Figure B).



**Figure A
Immunoblot Analysis**
Lysates from untreated (Lanes 1 and 3) and PDGF treated (Lanes 2 and 4) 3T3/NIH cells were resolved by electrophoresis, transferred to nitrocellulose and probed with anti-phospho-Akt1/PKB α (Thr308) (1:2,500) (Lanes 1 and 2) or total anti-Akt1/PKB α (Catalog # 07-416) (1:1,000) (Lanes 3 and 4). Proteins were visualized using a goat anti-rabbit secondary antibody conjugated to HRP and a chemiluminescence detection system. Arrow indicates phosphorylated Akt1/PKB α (~60kDa).

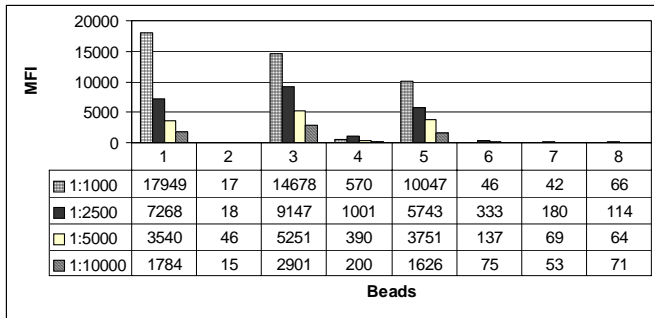


Figure B
Beadlyte® Specificity Assay

1:1,000 to 1:10,000 dilutions of this lot were incubated with a cocktail of microspheres conjugated to the following:

1. immunizing peptide containing phospho-Thr308
2. unmodified peptide, containing Thr308
3. recombinant Akt1, phosphorylated
4. recombinant Akt1, unphosphorylated
5. recombinant Akt2, phosphorylated
6. recombinant Akt2, unphosphorylated
7. recombinant Akt3, phosphorylated
8. recombinant Akt3, unphosphorylated

Unbound antibody was removed by filtration. Bound antibody complexes were detected with a biotin conjugated anti-rabbit secondary antibody followed by a phycoerythrin-streptavidin conjugate. Fluorescence was read on a Luminex® 100™ instrument. Median Fluorescent Intensity (MFI) is plotted.

Produced in collaboration with Epitomics, Inc.

General References:

1. Alessi D.R., *et al.*, *EMBO* **15**: 6541-6551, 1996.
2. Cross, D.A., *et al.*, *Nature* **378**: 785-789, 1995.
3. James, S.R., *et al.*, *Biochem. J.* **315**: 709-713, 1996.
4. Burgering and Coffey, *Nature* **376**: 599-601, 1995.
5. Ackler, S., *et al.*, *Oncogene* **21**: 198-206, 2002.

Immunoblot Protocol

1. Perform SDS-polyacrylamide gel electrophoresis (SDS-PAGE) on a cell lysate sample (cell lysis buffer: 50mM Tris-HCl, pH 7.4; 1% NP-40; 0.25% sodium deoxycholate; 150mM NaCl; 1mM EDTA; 1mM PMSF; 1µg/ml each aprotinin, leupeptin, pepstatin; 1mM Na₃VO₄; 1mM NaF) and transfer the proteins to nitrocellulose. Wash the blotted nitrocellulose twice with water.
2. Block the blotted nitrocellulose in freshly prepared TBS containing 3% nonfat dry milk (Catalog # 20-200) and 0.05% Tween®-20 (TBST-MLK) for 1 hour at room temperature with constant agitation.
3. Incubate the nitrocellulose with a **1:1,000-1:5,000 dilution of anti-phospho-Akt1/PKBα (Thr308), clone NL50**, diluted in freshly prepared TBST-MLK overnight with agitation at 4°C.
4. Wash the nitrocellulose twice with water.
5. Incubate the nitrocellulose in the secondary reagent of choice (a **goat anti-rabbit HRP conjugated IgG**, Catalog # 12-348, 1:5000 dilution was used) in TBST-MLK for 1.5 hours at room temperature with agitation.
6. Wash the nitrocellulose twice with water.
7. Wash the nitrocellulose in TBS-0.1% Tween®-20 for 3-5 minutes.
8. Rinse the nitrocellulose in 2-3 changes of water, 4-5 minutes each wash.
9. Use detection method of choice (enhanced chemiluminescence was used).