

Certificate of Analysis

Product: Anti-AKT (Rabbit) Human/Mouse/Rat/Chicken Specific

Code: 100-401-401

Lot #: 17992

Size: 200 µl

Physical State: Liquid (sterile filtered)

Protein Concentration: 85 mg/ml (by Refractometry)

Buffer: 0.02 M Potassium Phosphate, 0.15 M Sodium Chloride, pH 7.2

Stabilizer: None

Preservative: 0.1% (w/v) Sodium Azide

Storage Conditions: Store vial at -20° C prior to opening. Aliquot contents and freeze at -20° C or below for extended storage. Avoid cycles of freezing and thawing. Centrifuge product if not completely clear after standing at room temperature. This product is stable for several weeks at 4° C as an undiluted liquid. Dilute only prior to immediate use. Expiration date one (1) year from opening.

Background Information: AKT is a component of the PI-3 kinase pathway and is activated by phosphorylation at Ser 473 and Thr 308. AKT is a cytoplasmic protein also known as Protein Kinase B (PKB) and rac (related to A and C kinases). AKT is a key regulator of many signal transduction pathways. AKT Exhibits tight control over cell proliferation and cell viability. Overexpression or inappropriate activation of AKT is noted in many types of cancer. AKT mediates many of the downstream events of PI 3-kinase (a lipid kinase activated by growth factors, cytokines and insulin). PI 3-kinase recruits AKT to the membrane, where it is activated by PDK1 phosphorylation. Once phosphorylated, AKT dissociates from the membrane and phosphorylates targets in the cytoplasm and the cell nucleus. AKT has two main roles: (i) inhibition of apoptosis; (ii) promotion of proliferation.

Application Note(s): This antibody is suitable for western blotting to detect a single band of the expected apparent molecular weight, immunohistochemistry (Formalin-fixed paraffin-embedded sections), immunofluorescence microscopy using paraformaldehyde-fixed primary cardiomyocyte cultures, immunoprecipitation, transfected cell culture, primary cell culture and ELISA. Researchers should determine optimal titers for applications that are not stated below.

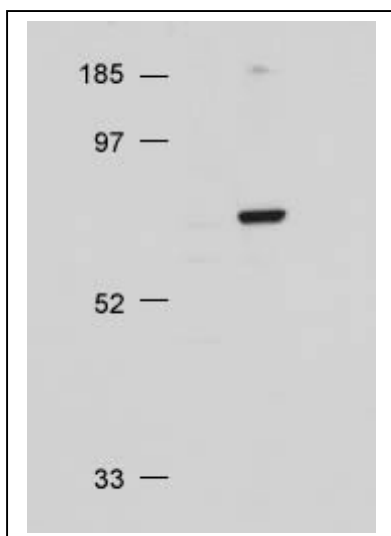


Figure 2. Immunoblotting. Rabbit anti-AKT was used at a 1:500 dilution to detect AKT by Western blot. Approximately 20 µg of an NIH/3T3 whole cell lysate was loaded on a 10% NuPage SDS-PAGE gel using MOPS buffer. After washing, a 1:10,000 dilution of HRP conjugated Gt-a-Rabbit IgG (611-103-122) preceded color development using Pierce Chemical's SuperSignal™ substrate.

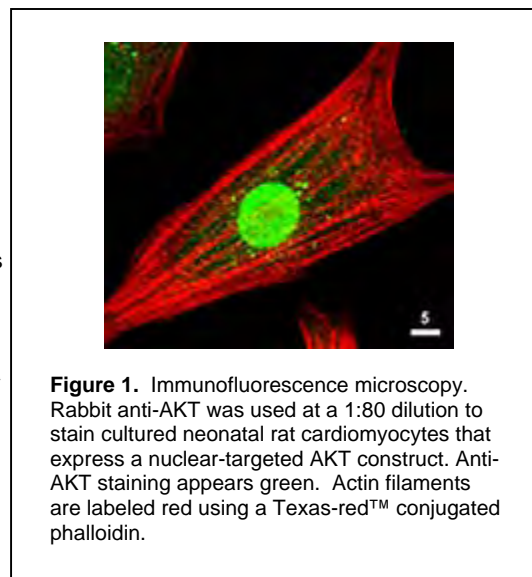
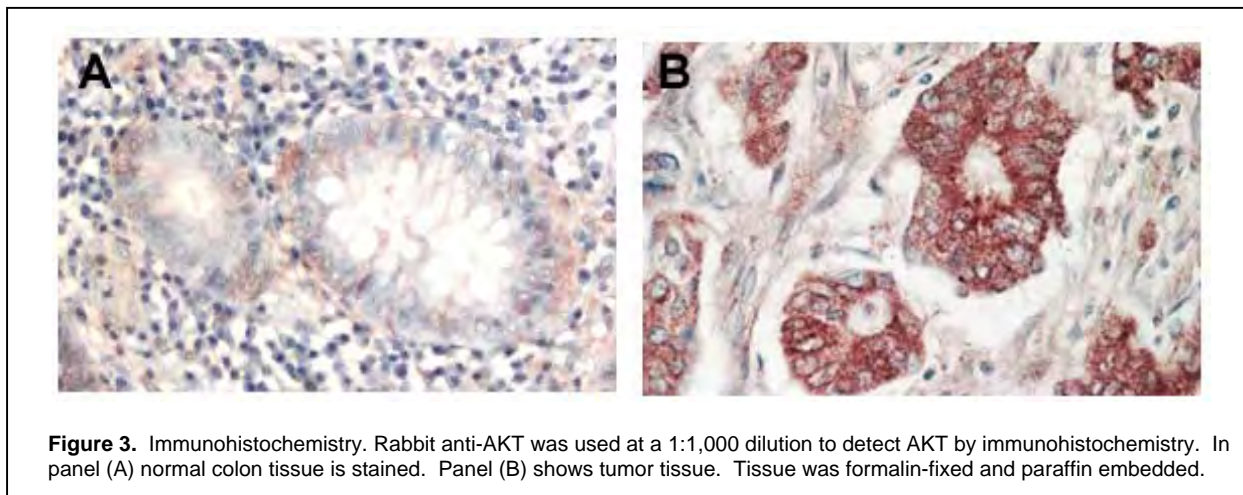


Figure 1. Immunofluorescence microscopy. Rabbit anti-AKT was used at a 1:80 dilution to stain cultured neonatal rat cardiomyocytes that express a nuclear-targeted AKT construct. Anti-AKT staining appears green. Actin filaments are labeled red using a Texas-red™ conjugated phalloidin.

Recommended Dilutions:

ELISA	1:2,000 - 1:10,000
WESTERN BLOT	1:500 - 1:2,000
IMMUNOHISTOCHEMISTRY	1:500 - 1:2,000
IF MICROSCOPY	1:100 - 1:1,000
OTHER APPLICATIONS	User Optimized

Purity and Specificity: This antiserum is directed against AKT and reacts with the AKT from human tissues. Based on sequence we expect this antibody to react as well with rat, mouse and chicken AKT.



Related Product(s):

#000-000-401	CONTROL PEPTIDE For Anti-AKT
#600-401-268	Anti-AKT phospho specific pS473 (RABBIT)
#600-401-269	Anti-AKT phospho specific pT308 (RABBIT)
#611-103-122	HRP Anti-Rabbit IgG [H&L] MX10 (GOAT)
#611-132-122	IRDye800 Anti-Rabbit IgG [H&L] MX10 (GOAT)

Immunogen: This whole rabbit serum was prepared by repeated immunizations with a synthetic peptide C-R-P-H-F-P-Q-F-S-Y-S-A-S-G-T-A corresponding to the C-terminus (460-480) of human, rat and mouse and chicken AKT proteins conjugated to KLH using maleimide. A residue of cysteine was added to the amino terminal end to facilitate coupling.

General References:

Lawlor, M. A. and Alessi, D.R. (2001). PKB/AKT: a key mediator of cell proliferation, survival and insulin responses. *J. Cell Science* **114**:2903-2910.

Alessi, D. R. (2001). Discovery of PDK1, one of the missing links in insulin signal transduction. *Biochem. Soc. Trans.* **29**,1 -14.

USDA Certification: All products of animal origin manufactured by Rockland Immunochemicals are derived from starting materials of North American origin. Collection was performed in United States Department of Agriculture (USDA) inspected facilities and all materials have been inspected and certified to be free of disease and suitable for exportation.

Note: This product is for research use only and is not intended for therapeutic or diagnostic applications. Please contact a technical service representative for more information.

