

Datasheet for 200-342-269

## AKT phospho T308 DyLight 549 Conjugated Antibody

### Overview

|                      |                                                                                  |
|----------------------|----------------------------------------------------------------------------------|
| <b>Description:</b>  | Anti-AKT pT308 (MOUSE) Monoclonal Antibody DyLight™ 549 Conjugated - 200-342-269 |
| <b>Item No.:</b>     | 200-342-269                                                                      |
| <b>Size:</b>         | 100 µg                                                                           |
| <b>Applications:</b> | WB                                                                               |
| <b>Reactivity:</b>   | Human, Mouse, Rat, Monkey                                                        |
| <b>Host Species:</b> | Mouse                                                                            |

### Product Details

|                      |                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                        |
|----------------------|----------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|
| <b>Background:</b>   | 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 AKT1, 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. |
| <b>Synonyms:</b>     | mouse anti-AKT pT308 DyLight™549 conjugated Antibody, DyLight™ 549 conjugated mouse anti-AKT pT308 Antibody, RAC-PK-alpha, Protein kinase B, PKB, C-AKT, RAC-alpha serine/threonine-protein kinase, Proto-oncogene c-Akt, AKT1, AKT 1, AKT-1                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                           |
| <b>Host Species:</b> | Mouse                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                  |
| <b>Conjugate:</b>    | DyLight™ 549                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                           |
| <b>Clonality:</b>    | Monoclonal                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                             |
| <b>Clone ID:</b>     | 18F3.H11                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                               |
| <b>Format:</b>       | IgG1                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                   |
| <b>F/P Ratio:</b>    | 4.0                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                    |

## Target Details

|                            |                                                                                                                                                                                                                                                                                                                                                                                                                                                                                 |
|----------------------------|---------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|
| <b>Gene Name:</b>          | AKT1                                                                                                                                                                                                                                                                                                                                                                                                                                                                            |
| <b>Reactivity:</b>         | Human, Mouse, Rat, Monkey                                                                                                                                                                                                                                                                                                                                                                                                                                                       |
| <b>PTM Specificity:</b>    | Phosphorylation                                                                                                                                                                                                                                                                                                                                                                                                                                                                 |
| <b>Immunogen Type:</b>     | Conjugated Peptide                                                                                                                                                                                                                                                                                                                                                                                                                                                              |
| <b>Immunogen:</b>          | This monoclonal antibody was produced by repeated immunizations with a synthetic phosphopeptide corresponding to residues surrounding T308 of human AKT1 protein.                                                                                                                                                                                                                                                                                                               |
| <b>Purity/Specificity:</b> | This product was purified from concentrated tissue culture supernate by Protein A chromatography. This antibody is specific for human and mouse AKT protein phosphorylated at T308. A BLAST analysis was used to suggest cross-reactivity with AKT pT308 from most vertebrate species sources based on 100% homology with the immunizing sequence. Cross-reactivity with AKT from other sources has not been determined. Cross-reactivity with AKT2 and AKT3 will likely occur. |
| <b>Relevant Links:</b>     | <ul style="list-style-type: none"> <li>• <a href="#">UniProtKB - P31749</a></li> <li>• <a href="#">NCBI - 62241011</a></li> <li>• <a href="#">GenelD - 207</a></li> </ul>                                                                                                                                                                                                                                                                                                       |

## Application Details

|                             |                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                         |
|-----------------------------|-------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|
| <b>Tested Applications:</b> | WB                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                      |
| <b>Application Note:</b>    | This monoclonal antibody is tested in western blotting. This antibody is suitable for immunofluorescence microscopy, IHC, FLISA, and flow cytometry. Expect a band approximately 56 kDa in size corresponding to phosphorylated AKT protein by western blotting in the appropriate cell lysate or extract. This phospho-specific monoclonal antibody reacts with human and mouse AKT pT308 and shows minimal reactivity by ELISA against the non-phosphorylated form of the immunizing peptide. Specific conditions for reactivity should be optimized by the end user. |
| <b>Assay Dilutions:</b>     | All assays should be optimized by the user. Recommended dilutions (if any) may be listed below.                                                                                                                                                                                                                                                                                                                                                                                                                                                                         |
| <b>ELISA:</b>               | 1:4,000 - 1:20,000                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                      |
| <b>FC:</b>                  | User Optimized                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                          |
| <b>FLISA:</b>               | >1:20,000                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                               |
| <b>IF:</b>                  | >1:5,000                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                |
| <b>IHC:</b>                 | 20 µg/mL                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                |

WB: >1:10,000

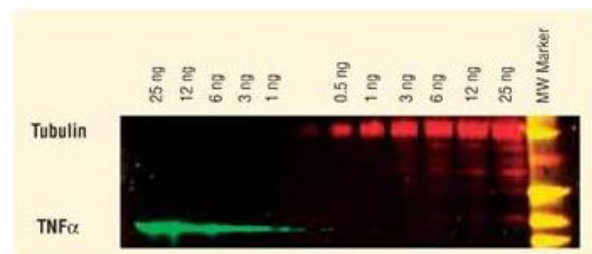
## Formulation

|                        |                                                                        |
|------------------------|------------------------------------------------------------------------|
| Physical State:        | Lyophilized                                                            |
| Concentration:         | 1.0 mg/mL by UV absorbance at 280 nm                                   |
| Buffer:                | 0.02 M Potassium Phosphate, 0.15 M Sodium Chloride, pH 7.2             |
| Preservative:          | 0.01% (w/v) Sodium Azide                                               |
| Stabilizer:            | 10 mg/mL Bovine Serum Albumin (BSA) - Immunoglobulin and Protease free |
| Reconstitution Volume: | 100 µL                                                                 |
| Reconstitution Buffer: | Restore with deionized water (or equivalent)                           |

## Shipping & Handling

|                     |                                                                                                                                                                                                                                                                                                                                                 |
|---------------------|-------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|
| Shipping Condition: | Ambient                                                                                                                                                                                                                                                                                                                                         |
| Storage Condition:  | Store vial at 4° C prior to restoration. For extended storage aliquot contents and freeze at -20° C or below. 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:         | Expiration date is one (1) year from date of receipt.                                                                                                                                                                                                                                                                                           |

## Images



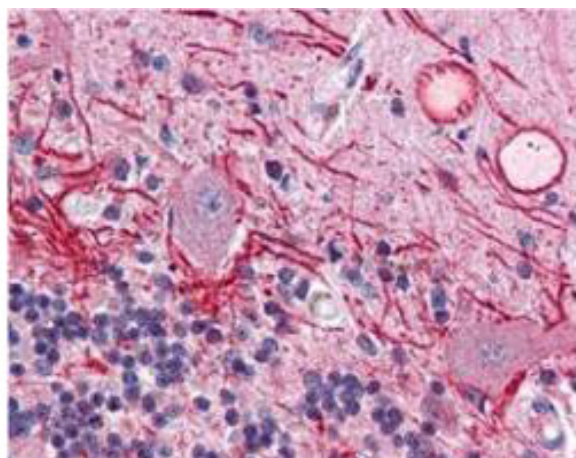
### Western Blot

DyLight™ dyes can be used for two-color western blot detection with low background and high signal. Anti-tubulin was detected using a DyLight™ 549 conjugate. Anti-TNFα was detected using a DyLight™ 649 conjugate. The image was captured using the Typhoon™ 9410 Imaging System.

| Emission      | Color                                                                             | DyLight™ Dye | Ex/Em (nm) | $\epsilon$ (M <sup>-1</sup> cm <sup>-1</sup> ) | Similar Dyes                       |
|---------------|-----------------------------------------------------------------------------------|--------------|------------|------------------------------------------------|------------------------------------|
| Blue          |  | 405          | 400/420    | 30,000                                         | Alexa™ 405, Cascade Blue           |
| Green         |  | 488          | 493/518    | 70,000                                         | Alexa™ 488, Cy2®, FITC             |
| Yellow        |  | 549          | 550/568    | 150,000                                        | Alexa™ 546, Alexa 555, Cy3®, TRITC |
| Red           |  | 649          | 646/674    | 250,000                                        | Alexa™ 647, Cy5®                   |
| Near Infrared |  | 680          | 682/715    | 140,000                                        | Alexa™ 680, Cy5.5®, IRDye™ 700     |
| Infrared      |  | 800          | 770/794    | 270,000                                        | IRDye™ 800                         |

### Diagram

Properties of DyLight™ Fluorescent Dyes.

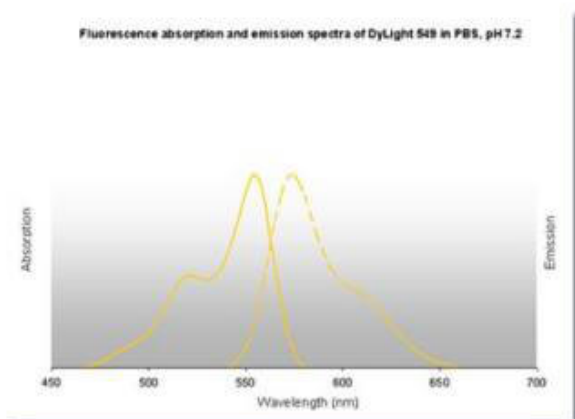


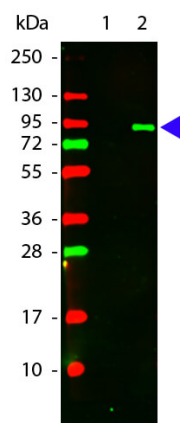
### Immunohistochemistry

Immunohistochemistry of Mouse anti-AKT pT308 antibody.  
 Tissue: human brain cerebellum tissue (40X). Fixation: formalin fixed paraffin embedded. Antigen retrieval: not required. Primary antibody: AKT pT308 antibody at 20 µg/mL for 1 h at RT. Secondary antibody: Peroxidase rabbit secondary antibody at 1:10,000 for 45 min at RT. Localization: staining of Purkinje neurons and cell processes in the cerebellum, cytosolic as well as occasionally nuclear. Staining: AKT pT308 as precipitated red signal with hematoxylin purple nuclear counterstain.

### Diagram

DyLight™ 549 Fluorescence absorption and emission spectra.





#### Western Blot

Western Blot of Mouse anti-AKT pT308 antibody DyLight 549 Conjugated. Lane 1: GST-AKT1 Unactive recombinant protein. Lane 2: GST-AKT1 Active recombinant protein. Load: 50 ng per lane. Primary antibody: None. Secondary antibody: DyLight 549 conjugated Ms-a-AKT pT308 (p/n 200-342-269) was used at a 1:1,000 dilution for 1 h at RT. Block: 3% BSA (p/n BSA-30) in TBS for 30 min at RT. Predicted/Observed size: (indicated by arrowhead at ~79 kDa). Other band(s): None.

## Disclaimer

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. 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. All properties listed are typical characteristics and are not specifications. All suggestions and data are offered in good faith but without guarantee as conditions and methods of use of our products are beyond our control. All claims must be made within 30 days following the date of delivery. The prospective user must determine the suitability of our materials before adopting them on a commercial scale. Suggested uses of our products are not recommendations to use our products in violation of any patent or as a license under any patent of Rockland Immunochemicals, Inc. If you require a commercial license to use this material and do not have one, then return this material, unopened to: Rockland Inc., P.O. BOX 5199, Limerick, Pennsylvania, USA.