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Datasheet for KCAE75

# AKT3 Western Chemiluminescent Blotting Kit

## Overview

| | |
|---|---|
| **Description:** | AKT3 Western Blot Kit - KCAE75 |
| **Item No.:** | KCAE75 |
| **Size:** | 1 Kit |
| **Applications:** | IF, WB |
| **Reactivity:** | Human |
| **Host Species:** | Mouse |

## Product Details

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|---|---|
| **Background:** | Rockland Immunochemicals' Chemiluminescent Western Blot Kit for AKT3 combines all of the necessary reagents with a rapid proven protocol and the extremely high signal detection of our luminol chemiluminescent substrate for the detection of native and/or recombinant human AKT3 protein. AKT3 Western Blotting kit allows for the detection of endogenous protein levels of human AKT3 present in cell lysates provided by the user. As a positive control, this kit includes a MDA-MB468 whole cell lysate proven to contain AKT3. After protein separation by SDS-PAGE and transfer, the membrane is probed with Rockland's optimized Anti-AKT3 antibody. Detection of the membrane bound antibody-antigen complex is achieved by the addition of a secondary antibody conjugated to the enzyme horseradish peroxidase. The enzyme reacts with a specialized formulation of luminol, an extremely sensitive, non-radioactive substrate that emits light and allows visualization using X-ray film or other imaging methods, including highly sensitive CCD cameras and imaging systems. AKT3 Western Blotting is ideal for investigators involved in Cell Signaling, Cancer, Neuroscience and Signal Transduction research. |
| **Synonyms:** | AKT 3 western blotting, AKT-3, PKB antibody, PKB gamma antibody, PKBGAMMA antibody, PRKBG antibody, Protein kinase Akt 3 antibody, Protein kinase B gamma antibody, RAC-gamma serine/threonine-protein kinase, RAC-PK-gamma, western bloting kit, Chemiluminescent WB, Western Blotting Kit, Chemiluminescent Kit, Peroxidase Kit, Immunoblotting Kit |
| **Host Species:** | Mouse |
| **Clonality:** | Monoclonal |
| **Clone ID:** | 9A5.H9.G7 |
| **Format:** | IgG1 |
| **Detection Kit Type:** | Chemiluminescent Western Blot Kit |

## Target Details

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|---|---|
| **Gene Name:** | AKT3 |
| **Reactivity:** | Human |
| **Purity/Specificity:** | The kit is designed to detect both unphosphorylated and phosphorylated forms of the protein. Cross reactivity with AKT3 from other species has not been determined, however, the sequence of the immunogen shows 100% identity to human, mouse, and rat, therefore, cross reactivity is expected. Cross-reactivity with AKT1 and AKT2 has not been determined. This kit contains sufficient substrate for up to 5 mini blots at 7.5 x 8 cm2 (1,800 cm2) and is stable for at least 1 year when stored as indicated. |
| **Relevant Links:** | • GeneID - 10000<br>• UniProtKB - Q9Y243<br>• NCBI - NP_001193658.1<br>• AKT3 Chemiluminescent WBlot Kit Protocol |

## Application Details

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|---|---|
| **Tested Applications:** | IF, WB |
| **Application Note:** | Use Rockland Immunochemicals' Anti-AKT3 Chemiluminescent Kit for Western Blotting for detection of human AKT3 native and recombinant proteins by western blot. Expect a band approximately 56 kDa in size corresponding to AKT3 protein. This kit is useful for both western blotting and dot blotting methods. Please read the entire product insert prior to use. |
| **Assay Dilutions:** | All assays should be optimized by the user. Recommended dilutions (if any) may be listed below. |
| **WB:** | User Optimized |

## Formulation

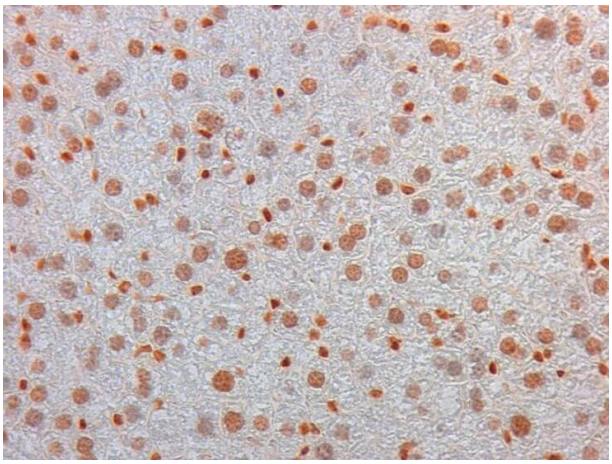
| | |
|---|---|
| **Physical State:** | n/a |
| **Preservative:** | Wash buffers MUST NOT contain SODIUM AZIDE or other inhibitors of peroxidase activity! |
| **Stabilizer:** | None |

## Shipping & Handling

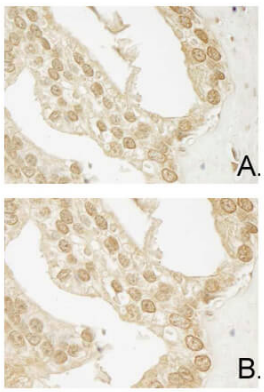
| | |
|---|---|
| **Shipping Condition:** | Dry Ice |

| | |
|---|---|
| **Storage Condition:** | See kit insert for complete instructions. |
| **Expiration:** | See kit insert for complete instructions. |

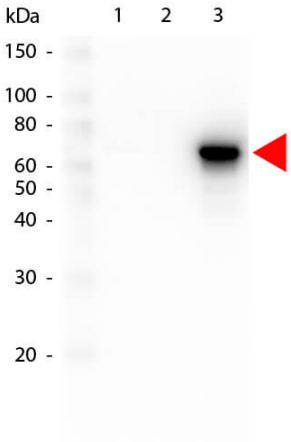
## Images



**Immunohistochemistry**
Immunohistochemistry of Mouse Monoclonal anti AKT3 Antibody in Mouse Embryonic Kidney. Tissue: Mouse Liver. Fixation: FFPE buffered formalin 10% conc. Ag Retrieval: Heat, Citrate pH 6.2. Pressure Cooker. Primary antibody: anti-AKT3 at 2ug/ml for 1.5 hour @ room Temp. Secondary Ab: MOUSE ON MOUSE HRP POLYMER 45" RT.

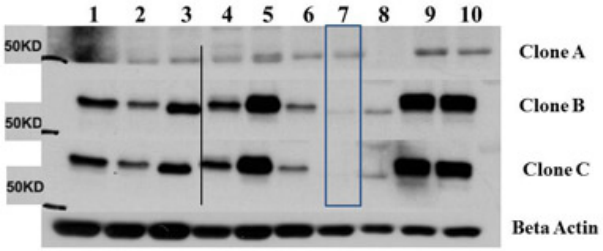



**Immunohistochemistry**
Immunohistochemistry of Mouse Anti-AKT3 antibody. Tissue: human prostate carcinoma. A) AKT-3 antibody produced using CELLine, B) AKT-3 antibody produced using roller bottle. Fixation: formalin fixed paraffin embedded. Antigen retrieval: not required. Primary antibody: AKT-3 antibody at 10 µg/mL for 1 h at RT. Secondary antibody: Peroxidase mouse secondary antibody at 1:10,000 for 1 h at RT. Localization: AKT3 is nuclear and occasionally cytoplasmic. Staining: AKT3 as precipitated brown signal with hematoxylin purple nuclear counterstain.



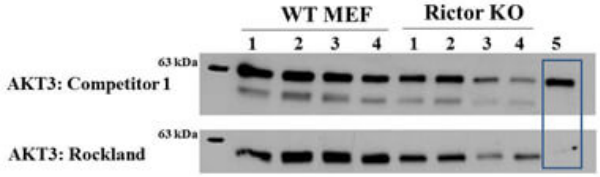

**Western Blot**

Western Blot of AKT isoform specific antibodies: 50 ng of recombinant GST-AKT1, -AKT2 and -AKT3 proteins were separated by SDS PAGE (4-20% gel) and transferred onto 0.2 µm nitrocellulose. Lane 1: GST Tagged recombinant AKT1. Lane 2: GST Tagged recombinant AKT2. Lane 3: GST Tagged recombinant AKT3. The membrane was blocked with Blocking Buffer (p/n MB-070) for 1 h at room temperature. Mouse anti-AKT1 (p/n 200-301-E75) at a dilution of 1 µg/mL in Blocking Buffer and incubated for 16 h at 4°C. Rabbit anti-Mouse IgG HRP (p/n 610-403-C46) secondary antibodies were applied at 0.05 µg/mL in Blocking Buffer and incubated for 1 h at room temperature followed by detection with FemtoMax™ chemiluminescent reagent (p/n FEMTOMAX-110). Predicted/Observed size: The expected size for all recombinant GST-AKT isoforms is 85 kDa. Arrowheads indicate the position of AKT isoform.




**Western Blot**

Western Blot of Mouse Anti-AKT3 antibody. Lane 1: C2C12. Lane 2: MEF#1. Lane 3: MEF#2. Lane 4: A549. Lane 5: Calu-1. Lane 6: PC3. Lane 7: HepG2. Lane 8: Jurkat. Lane 9: SKOV3. Lane 10: 293T. Load: 35 µg per lane. Primary antibody: AKT-3 antibody at 1:1000 for overnight at 4°C. Secondary antibody: Anti mouse secondary antibody at 1:20,000 for 1 h at RT. Block: 5% BLOTTO overnight at 4°C. Predicted/Observed size: 56 kDa for AKT3.




**Western Blot**

Western Blot of Mouse anti-AKT3 antibody. Lane 1: Control. Lane 2: Rapa. Lane 3: T50. Lane 4: T250. Lane 5: Control. Lane 6: Rapa. Lane 7: T50. Lane 8: T250. Lane 9: AKT3 null. Load: 35 µg per lane. Primary antibody: AKT-3 antibody at 1:1000 for overnight at 4°C. Secondary antibody: Anti mouse secondary antibody at 1:20,000 for 1 h at RT. Block: 5% BLOTTO overnight at 4°C. Predicted/Observed size: 56 kDa for AKT3.

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