Revision 1

Store at -20C

#14702

Akt (E17K Mutant Specific) (D1T7P) Rabbit mAb

Cell Signaling TECHNOLOGY®

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For Research Use Only. Not for Use in Diagnostic Procedures.

Applications:	Reactivity:	Sensitivity:	MW (kDa):	Source/Isotype:	UniProt ID:	Entrez-Gene Id:
W, IP, IF-IC	H	Transfected Only	87 (Akt-GFP)	Rabbit IgG	#P31751, #Q9Y243, #P31749	208, 10000, 207

Product Usage Information

Application	Dilution
Western Blotting	1:1000
Immunoprecipitation	1:50
Immunofluorescence (Immunocytochemistry)	1:800

Storage

Supplied in 10 mM sodium HEPES (pH 7.5), 150 mM NaCl, 100 µg/ml BSA, 50% glycerol and less than 0.02% sodium azide. Store at –20°C. Do not aliquot the antibody.

Specificity/Sensitivity

Akt (E17K Mutant Specific) (D1T7P) Rabbit mAb recognizes levels of transfected E17K mutant Akt1 and Akt2 protein. This antibody weakly recognizes transfected E17K mutant Akt3.

Species predicted to react based on 100% sequence homology

Mouse, Rat

Source / Purification

Monoclonal antibody is produced by immunizing animals with a synthetic peptide corresponding to E17K mutant sequence of human Akt1 protein.

Background

Akt, also referred to as PKB or Rac, plays a critical role in controlling cell survival and apoptosis (1-3). This protein kinase is activated by insulin and various growth and survival factors to function in a wortmannin-sensitive pathway involving PI3 kinase (2,3). Akt is activated by phospholipid binding and activation loop phosphorylation at Thr308 by PDK1 (4) and by phosphorylation within the carboxy terminus at Ser473. The previously elusive PDK2 responsible for phosphorylation of Akt at Ser473 has been identified as mammalian target of rapamycin (mTOR) in a rapamycin-insensitive complex with rictor and Sin1 (5,6). Akt promotes cell survival by inhibiting apoptosis through phosphorylation and inactivation of several targets, including Bad (7), forkhead transcription factors (8), c-Raf (9), and caspase-9. PTEN phosphatase is a major negative regulator of the PI3K/Akt signaling pathway (10). LY294002 is a specific PI3 kinase inhibitor (11). Another essential Akt function is the regulation of glycogen synthesis through phosphorylation and inactivation of GSK-3α and β (12,13). Akt may also play a role in insulin stimulation of glucose transport (12). In addition to its role in survival and glycogen synthesis, Akt is involved in cell cycle regulation by preventing GSK-3β-mediated phosphorylation and degradation of cyclin D1 (14) and by negatively regulating the cyclin-dependent kinase inhibitors p27 Kip1 (15) and p21 Waf1/Cip1 (16). Akt also plays a critical role in cell growth by directly phosphorylating mTOR in a rapamycin-sensitive complex containing raptor (17). More importantly, Akt phosphorylates and inactivates tuberin (TSC2), an inhibitor of mTOR within the mTOR-raptor complex (18,19).

Mutation of the glutamic acid at residue 17 to lysine (E17K) of Akt was initially identified in human breast, colorectal, and ovarian cancers (20). This conserved glutamic acid residue is located at the lipid-binding pocket of the Akt1 plextrin homology domain. The E17K mutation increases the affinity between Akt1 and phospholipids at the plasma membrane, leading to increased Akt1 recruitment, super-activation of the Akt pathway, cellular transformation, and tumor formation (20,21). Additional studies detect the presence of the Akt1 (E17K) mutation in multiple cancers, including lung cancer, prostate cancer, and endometrial carcinoma (22,23). The presence of mutant Akt3 (E17K) protein has also been seen in cases of melanoma (24).

Background References

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Species Reactivity

Species reactivity is determined by testing in at least one approved application (e.g., western blot).

Western Blot Buffer

IMPORTANT: For western blots, incubate membrane with diluted primary antibody in 5% w/v BSA, 1X TBS, 0.1% Tween® 20 at 4°C with gentle shaking, overnight.

Applications Key

W: Western Blotting **IP:** Immunoprecipitation **IF-IC:** Immunofluorescence (Immunocytochemistry)

Cross-Reactivity Key

H: Human

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Limited Uses