

Human AKT1 ProtéGene™ Set

Catalog# A1010
Lot# Labeled on vial

Materials Provided

1. pMEV2HA-AKT1-WT (A1010a): 20µg in 40µl TE, 0.5mg/ml.
2. pMEV2HA-AKT1-K179A (A1010b): 20µg in 40 µl TE, 0.5mg/ml.
3. pMEV2HA-AKT1-DD (A1010c): 20µg in 40µl TE, 0.5mg/ml.
4. pMEV2HA-AKT1-AA(A1010d): 20µg in 40µl TE, 0.5mg/ml.
5. pMEV2HA-AKT1-KAAA(A1010e): 20µg in 40µl TE, 0.5mg/ml.
6. Product Information Sheet.

Note: Individual plasmids can be ordered separately. Some plasmids are shipped as lyophilized pellet.

Receiving and Storage:

If received in lyophilized form, add 40µl sterile DI water to the vial, mix thoroughly by vortex and then collect the contents by centrifuging the vials briefly in a microcentrifuge. If received in liquid form, spin the vials briefly in a microcentrifuge to collect the contents. Store the products at 2-8°C if used immediately or, store at -20°C for extended storage.

Prokaryotic selection:

The kanamycin-resistance gene (aminoglycoside 3' phosphotransferase) expression cassette in the plasmids confers Kanamycin resistance to bacteria cells. Bacterial cells transformed with the plasmids should be maintained and grown in media containing 25-50µg/ml Kanamycin (e.g.cat#LK-1100, Pre-Poured LB Agar plates, Biomyx, San Diego, California).

Eukaryotic selection:

The neomycin resistance gene, driven by SV40 early promoter, confers G418 resistance to eukaryotic cells. Stable mammalian cell lines can be selected with G418.

Description of AKT1 and Mutants

AKT1^[1] and the related AKT2 are serine-threonine protein kinases activated by many growth factors including PDGF, EGF, FGF, IGF-1 and insulin, as well as protein phosphatase inhibitors. This activation is rapid and specific and is mediated by phosphatidylinositol 3-kinase. The activation of AKT1 has also been shown to be essential in the suppression of apoptosis by many of the survival growth factors.

Activation of AKT1 requires the phosphorylation on both Thr-308 and Ser-473^[2,3,4]. AKT1 can not be activated if either T308 or S473 was changed to an alanine residue. If both T308 and S473 are changed to an aspartic acid residue, however, the kinase become constitutively active. That is, it can activate downstream signaling pathways without any upstream activators.^[3,4] K179 is an essential residue for catalysis and, hence the mutant K179A (A1010b) no longer has protein kinase activity. K179A, T308A/S473A and the mutant with all three mutations (P1010e) have been shown to inhibit the signaling pathways when transfected into mammalian cells that have endogenous AKT1 proteins (i.e. dominant negative effects). These mutants can also been used to identify upstream activators, or to study the phosphorylation patterns of AKT1 *in vivo* and *in vitro*.

Molecular Features of the inserts:

Gene: Homo sapiens v-akt murine thymoma viral oncogene homolog 1 (AKT1), transcript variant 1

Other Names: PKB, RAC, PRKBA, MGC99656, RAC-ALPHA

GenBank Reference Sequence: NM_005163

5'-Cloning Site: Bam HI

5'-Junction Sequence: 5'... tagctt **ggatcc ATG** (AKT1)...3'

3'-Cloning Site: Kpn I

3'-Junction Sequence: 5' **ggtaccacgcgtattaatcc**
TCA (AKT1) ...3'

Human AKT1 Nucleotide and Protein Sequence

(1442 bps encoding 480 amino acid residues, with mutation locations marked in red)

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1 atgagcgacy ttgcttattt gaaggagggt tggctgcaca aacgaggggaa gtatcaatcg
M S D V A I V K E G W L H K R G E Y I K
61 accttcggcc caegctactt cctctcaag aatgtatggca ctttcatttg ctcaacggag
T C R P R Y F L K N D G T F I G Y K E
121 cggccggagg atgtggacca acgtggggct cccctcaaca acttcttcgt gggcggatgc
R P Q D V D Q R E A P L N N F S V A Q C
181 cagctgtatc agacggggcc cggccggccc aacacctca tcatccgtc cttcgatgtt
Q L M K T E R P R P N T F I I R C L Q W
241 accatgtca tcgaaacggac ctccatgtc gagatctggc aggacgggaa ggaaatggaca
T T V I E R T F H V E T P E E R E E W T
301 accggcatcc aagatgtggc tgacggccaa aagaacgggg aggaggaggaa gatggacttc
T A I Q T V A D G L K K Q E E E E M D F
361 cggatgggtt caccatgtt caacttggg gtcgaagaga tggatgttgc cttggccaag
R S G S P S D N S G A E E M E V S L A K
421 cccaaacggacc gcgttgcattt gaacggatgtt gatgtatggc agctgtctgg caaggccatc
P K H R V T M N E F Y E L K L G K G T
481 ttccggcaagg tgatgttgcattt gaacggatgtt gatgtatggc agctgtctgg catgAAGtc
F G K V I L V K E K A T G R Y Y A M K I
541 ctcaaaaggaa aagttcatgtc ggccaaaggac gatgttgcattt ccacacttcac cgagaaacgc
L K K E V I V A K D E V A H T L T E N R
601 gtctctggca acttcaggccatccatgttgcattt ccacacttcac agtacttcac ttccggccac
V L Q N S R H P F L T A L K Y S F Q T H
661 gaccgcgtt ctgttgtcat ggtatgcggc aacggggggc agctgtttttt ccacctgtcc
D R L C F V M E A N G G E L F F H L S
721 cgggggggtt tggttctccga ggacggggcc cgcttctatg ggcgtcgatgt tggttgcggc
C R E R V F S E D P A R F Y G A E I V S A
781 ctggactacc tgcacccgtt gaaacatgtt gtttacgggg acctcaatgtt ggaaacccctc
L D Y L H S C K N V V Y R D L K L E N L
841 atgttggacca agggatggccatccatgttgcattt ccacacttcac agtacttcac ttccggccac
M L D K D G H I K I T D F G L C K E G I
901 aaggacgtgtt caccatgttgcattt ccacacttcac agtacttcac ttccggccac
D G A A T M K T F C G T P E Y L A P E V
961 ctggaggacca atgtatccgtt cgttgcgtt gacttgggg ggttgcggcgtt ggtatgtac
L E D N D Y G R A V D W W G L G V V M Y
1021 gagatgttgcgttgcgttgcgttgcgttgcgttgcgttgcgttgcgttgcgttgcgttgcgttgcgtt
E M M C G R L P F Y N Q D H E K L F E L
1081 atccatgttgcgttgcgttgcgttgcgttgcgttgcgttgcgttgcgttgcgttgcgttgcgttgcgtt
I L M E I R F P R T L P G P E A K S L L
1141 tcagggtgttgcgttgcgttgcgttgcgttgcgttgcgttgcgttgcgttgcgttgcgttgcgttgcgtt
S G L L K K D P K Q R L G G G S E D A K
1201 gagatgttgcgttgcgttgcgttgcgttgcgttgcgttgcgttgcgttgcgttgcgttgcgttgcgttgcgtt
E I M Q H R F P A G I V W Q H V Y E K K
1261 ctggccggccatccatgttgcattt ccacacttcac agtacttcac ttccggccac
L S P P F K P Q V T S E T D T R Y F D E
1321 gagttcacggccatccatgttgcattt ccacacttcac agtacttcac ttccggccac
E F T A Q M I T I T P P D Q D D S M E C
1381 gtggacacggccatccatgttgcattt ccacacttcac agtacttcac ttccggccac
V D S E R R P H F P Q F S Y S A S G T A
1441 tga -

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Mutations:

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|----------------------|---|
| AKT1-WT (A1010a): | No mutation |
| AKT1-K179A (A1010b): | K179A: AAG → GCG |
| AKT1-DD (A1010c): | T308D: ACC → GAC; S473D: TCC → GAC. |
| AKT1-AA (A1010d): | T308A: ACC → GCC; S473A: TCC → GCC. |
| AKT1-KAAA (A1010e): | K179A: AAG → GCG; T308A: ACC → GCC;
S473A: TCC → GCC |

Selected References:

1. Staal, S. P., 1987. Molecular cloning of the akt oncogene and its human homologues AKT1 and AKT2: amplification of AKT1 in a primary human gastric adenocarcinoma. *Proc. Nat. Acad. Sci.* 84: 5034-5037,
2. Fayard E, Tintignac LA, Baudry A, Hemmings BA. 2005. Protein kinase B/Akt at a glance. *J Cell Sci.* Dec 15; 118(Pt 24):5675-8.
3. Ozes ON, Mayo LD, Gustin JA, Pfeffer SR, Pfeffer LM, Donner DB. NF-kappaB activation by tumour necrosis factor requires the Akt serine-threonine kinase. *Nature.* 1999; 401(6748):82-5.
4. Meier, R, Alessi DR, Cron P, Andjeklović M, Hemmings BA. 1997. Mitogenic Activation, phosphorylation, and nuclear translocation of protein kinase Bb. *J. Biol. Chem.*, 272: 30491-7.

Web Resources:

For sequence, references and a comprehensive description, please go to the links below or copy and paste the link to your browser address bar:

GeneBank Nucleotide Sequence:

OMIM gene description:

<http://www.ncbi.nlm.nih.gov/entrez/dispmim.cgi?id=164730>

Entrez Gene information page:

<http://www.ncbi.nlm.nih.gov/entrez/viewer.fcgi?db=nucleotide&val=62241010>