Technology
Biomyx
Innovation & Convenience for Life Sciences

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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^{0}C if used immediately or, store at –20^{0}C for extended storage.

Prokaryotic selection:

The kanamycin-resistance gene (aminoglcoside 3' phospho-transferase) 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, GFG, 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'… tacgct ggatcc **ATG** (AKT1)…3'
3'-Cloning Site: Kpn I
3'-Junction Sequence: 5' ggtaccacgcgtgattgaatcc 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 atgagcgacg tggctattgt gaaggagggt tggctgcaca aacgagggga gtacatcaag
    M  S  D   V  A  I   V  K  E  G   W  L  H   K  R  G   E  Y  I  K
 61 acctggcggc cacgctactt cctcctcaag aatgatggca ccttcattgg ctacaaggag
    T  W  R   P  R  Y   F  L  L  K   N  D  G   T  F  I   G  Y  K  E
121 cggccgcagg atgtggacca acgtgaggct cccctcaaca acttctctgt ggcgcagtgc
    R  P  Q   D  V  D   Q  R  E  A   P  L  N   N  F  S   V  A  Q  C
181 cagctgatga agacggagcg gccccggccc aacaccttca tcatccgctg cctgcagtgg
    Q  L  M   K  T  E   R  P  R  P   N  T  F   I  I  R   C  L  Q  W
241 accactgtca tcgaacgcac cttccatgtg gagactcctg aggagcggga ggagtggaca
    T  T  V   I  E  R   T  F  H  V   E  T  P   E  E  R   E  E  W  T
301 accgccatcc agactgtggc tgacggcctc aagaagcagg aggaggagga gatggacttc
    T  A  I   Q  T  V   A  D  G  L   K  K  Q   E  E  E   E  M  D  F
361 cggtcgggct cacccagtga caactcaggg gctgaagaga tggaggtgtc cctggccaag
    R  S  G   S  P  S   D  N  S  G   A  E  E   M  E  V   S  L  A  K
421 cccaagcacc gcgtgaccat gaacgagttt gagtacctga agctgctggg caagggcact
    P  K  H   R  V  T   M  N  E  F   E  Y  L   K  L  L   G  K  G  T
481 ttcggcaagg tgatcctggt gaaggagaag gccacaggcc gctactacgc catgAAGatc
    F  G  K   V  I  L   V  K  E  K   A  T  G   R  Y  Y   A  M  K  I
541 ctcaagaagg aagtcatcgt ggccaaggac gaggtggccc acacactcac cgagaaccgc
    L  K  K   E  V  I   V  A  K  D   E  V  A   H  T  L   T  E  N  R
601 gtcctgcaga actccaggca ccccttcctc acagccctga agtactcttt ccagacccac
    V  L  Q   N  S  R   H  P  F  L   T  A  L   K  Y  S   F  Q  T  H
661 gaccgcctct gctttgtcat ggagtacgcc aacgggggcg agctgttctt ccacctgtcc
    D  R  L   C  F  V   M  E  Y  A   N  G  G   E  L  F   F  H  L  S
721 cgggagcgtg tgttctccga ggaccgggcc cgcttctatg gcgctgagat tgtgtcagcc
    R  E  R   V  F  S   E  D  R  A   R  F  Y   G  A  E   I  V  S  A
781 ctggactacc tgcactcgga gaagaacgtg gtgtaccggg acctcaagct ggagaacctc
    L  D  Y   L  H  S   E  K  N  V   V  Y  R   D  L  K   L  E  N  L
841 atgctggaca aggacgggca cattaagatc acagacttcg ggctgtgcaa ggaggggatc
    M  L  D   K  D  G   H  I  K  I   T  D  F   G  L  C   K  E  G  I
901 aaggacggtg ccaccatgaa gACCttttgc ggcacacctg agtacctggc ccccgaggtg
    K  D  G   A  T  M   K  T  F  C   G  T  P   E  Y  L   A  P  E  V
961 ctggaggaca atgactacgg ccgtgcagtg gactggtggg ggctgggcgt ggtcatgtac
    L  E  D   N  D  Y   G  R  A  V   D  W  W   G  L  G   V  V  M  Y
1021 gagatgatgt gcggtcgcct gcccttctac aaccaggacc atgagaagct ttttgagctc
    E  M  M   C  G  R   L  P  F  Y   N  Q  D   H  E  K   L  F  E  L
1081 atcctcatgg aggagatccg cttcccgcgc acgcttggtc ccgaggccaa gtccttgctt
    I  L  M   E  E  I   R  F  P  R   T  L  G   P  E  A   K  S  L  L
1141 tcagggctgc tcaagaagga ccccaagcag aggcttggcg ggggctccga ggacgccaag
    S  G  L   L  K  K   D  P  K  Q   R  L  G   G  G  S   E  D  A  K
1201 gagatcatgc agcatcgctt ctttgccggt atcgtgtggc agcacgtgta cgagaagaag
    E  I  M   Q  H  R   F  F  A  G   I  V  W   Q  H  V   Y  E  K  K
1261 ctcagcccac ccttcaagcc ccaggtcacg tcggagactg acaccaggtg ttttgatgag
    L  S  P   P  F  K   P  Q  V  T   S  E  T   D  T  R   Y  F  D  E
1321 gagttcacgg cccagatgat caccatcaca ccacctgacc aagatgacag catggagtgt
    E  F  T   A  Q  M   I  T  I  T   P  P  D   Q  D  D   S  M  E  C
1381 gtggacagcg agcgcaggcc ccacttcccc cagttcTCCt actcggccag cggcacggcc
    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:

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, Andjeklovie 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/dispomim.cgi?id=164730
Entrez Gene information page:
http://www.ncbi.nlm.nih.gov/entrez/viewer.fcgi?db=nucleotide&val=62241010