

YAP2 ProteGene™ Set

Catalog# H1016
 Lot# 150071

Materials Provided

1. pMEV2HA-YAP2-WT (H1016a): 20 µg in 40 µl TE (0.5 mg/ml).
2. pMEV2HA-YAP2-5SA (H1016b): 20 µg in 40 µl TE (0.5 mg/ml).
3. pMEV2HA-YAP2-5SAΔC (H1016c): 20 µg in 40 µl TE (0.5 mg/ml).
4. Product Information Sheet.

Note: Individual plasmids can be ordered separately. Some plasmids are shipped as lyophilized pellet to increase the stability and/or reducing shipping cost.

Receiving and Storage:

If received in liquid form, spin the vials briefly in a microcentrifuge to collect the contents. If received in lyophilized form, add 40 µl sterile DI water to the vial, mix thoroughly by vortex and then collect the contents by centrifuging the vials briefly in a microcentrifuge. Store the products at 2-8°C if used immediately and 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 25-50 µg/ml Kanamycin (e.g. #LK-1100, Prepared 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 YAP2 and Mutants

Human YAP genes were clones initially using a chicken YAP as the probe (Sudol et al., 1995, Komuro et al. 2003). It is a coactivator of transcription factors. When phosphorylated by Lats or other upstream kinases, YAP protein translocates into the cytoplasm and has less transcriptional activation effect. YAP is one component of Hippo pathway involved in cell proliferation, apoptosis, cancer, cell contact inhibition and organ size control (Dong et al., 2007, Zhao et al., 2007).

YAP2 contains five HXRXXS motifs, which is the recognition sequence for upstream kinase Lats2. In YAP2-5SA mutant (H1016b), all five groups of serine residues (including S127) are mutated to alanine. This mutant protein can no longer be phosphorylated so more of it remains in the nucleus. As predicted, this mutant has relatively higher basal activity in a reporter assay (Zhao et al., 2007).

YAP2-5SA-ΔC (H1016c), which is the YAP2-5SA nucleus-localizing form with a deletion of the C-terminal transcription activation domain. This mutant YAP2 is insensitive to the Hippo pathway-induced cytoplasmic translocation and cannot activate gene expression, and therefore may act as a dominant-negative form. Overexpression of this mutant restored contact inhibition in a human cancer cell (Zhao et al., 2007).

Molecular Features of the inserts:

Gene: *Homo sapiens* yes-associated protein 2 (YAP2)

GenBank Accession#: AY316529

Synonyms: YAP; YAP2; YAP65; YKI

5'-Cloning Site: Bam HI

5'-Junction Sequence(upper strand) :

5'...tac gct **ATG GAT CCC**...3'

3'-Cloning Site: Sal I

3'-Junction Sequence (lower strand):

5'...gcccgg**gtcgac** CTA TAA CCA...3'

YAP2 Nucleotide & Amino Acid Sequences

(1467 bps, with mutated serine codons capitalized, in bold red and underlined. The major phosphorylation site S127 and the last amino acid Q291 in H1016c were highlighted as well.)

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1 atggatcccg ggcagcagcc gccgcctcaa ccggcccccc agggccaagg gcagccgctc
  M D P G Q Q P P P Q P A P Q G Q G Q P P
61 tcgcagcccc cgcaggggca gggcccgcgc tcggaccacc ggcaaccggc acccgcggcg
  S Q P P Q G Q G P P S G P G Q P A P A A
121 acccagpcgg cgcgcagggc acccccgcgc gggcatcaga tggcgacgt ccgcccggagc
  T Q A A P Q A P P A G H Q I V H V R G D
181 TCGgagacgg acctggaggc gctcttcaac gccgctatga accccaagac ggccaactgt
  S E T D L E A L F N A V M N P K T A N V
241 ccccagaccg tgcccctgag gctccggaa gctcccgaact ccttcttcaa gccgcccggag
  P Q T V P M R L R K L P D S F F K P P E
301 cccaaatccc actcccgaca ggccAGTact gatgcaggca ctgcaggagc cctgactcca
  P K S H S R Q A S T D A G T A G A L T P
361 cagcatgttc gagctcatTC CTCTccagct TCctgcagt tgggagctgt ttctctggg
  Q H V R A H S S P A S L Q L G A V S P G
421 acactgacc ccactggagt agtctctggc ccagagacta caccacagc tcagacttt
  T L T P T G V V S G P A A T P T A Q H L
481 cgacagTCCT CTtttgagat acctgatgat gtacctctgc cagcagggtg ggagatggca
  R Q S S F E I P D D V P L P A G W E M A
541 aagacatctt ctggtcagag atacttctta aatcacatcg atcacagcaac aacatggcag
  K T S S G Q R Y F L N H I D Q T T T W Q
601 gaccocagga aggcgatgct gtcccagatg aacgtcacag ccccaccag tccaccagt
  D P R K A M L S Q M N V T A P T S P P V
661 cagcagaata tgatgaactc ggcttcaggt cctcttctgt atggatggga acaagccatg
  Q Q N M M N S A S G P L P D G W E Q A M
721 actcagggat gagaaattta ctatataaac cataagaaca agaccacctc ttggctagac
  T Q D G E I Y Y I N H K N K T T S W L D
781 ccaagcgttg accctcgttt tgccatgaac cagagaatca gtcagatgac tccagtgaaa
  P R L D P R F A M N Q R I S Q S A P V K
841 cagccaccac cctggctcc ccagagccca CAGggaggcg tcatgggtgg cagcaactcc
  Q P P P L A P Q S P Q G G V M G G S N S
901 aaccagcagc aacagatgag actgcagcaa ctgcagatgg agaaggagag gctgcggctg
  N Q Q Q Q M R L Q Q L Q M E K E R L R L
961 aaacagcaag aactgcttcg gcagagagta gccctgcgta gccagttacc aacactggag
  K Q Q E L L R Q E L A L R S Q L P T L E
1021 caggatggtg ggaactcaaaa tccagtgctt tctcccggga tgtctcagga attgagaaca
  Q D G G T Q N P V S S P G M S Q E L R T
1081 atgacgacca atagctcaga tctcttctct aacagtggca cctatcactc tcgagatgag
  M T T N S S D P F L N S G T Y H S R D E
1141 agtacagaca gtggactaac catgagcagc tacagtgctc ctgcaaccoc agatgaactc
  S T D S G L S M S S Y S V P R T P D D F
1201 ctgaacagtg tggatgagat ggatacaggt gatactatca accaaagcac cctgcctcoa
  L N S V D E M D T G D T I N Q S T L P S
1261 cagcagaacc gtttcccaga ctacctgaa gccattctgt ggacaaatgt ggaccttgsa
  Q Q N R F P D Y L E A I P G T N V D L G
1321 acactggaag gagatggaat gaacatagaa ggagaggagc tgatgccaa gctgcaggaa
  T L E G D G M N I E G E E L M P S L Q E
1381 gctttgagtt ctgacatcct taatgacatg gactctgttt tggctgccac caagctagat
  A L S S D I L N D M E S V L A A T K L D
1441 aaagaaagct ttcttacatg gttatag
  K E S F L T W L -
  
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Mutations:

YAP2-WT (H1016a): No mutation.

YAP2-5SA (H1016b): S61A, S109A, S127A, S128A, S131A, S163A, S164A

YAP2-5SAΔC (H1016c): Mutations in H1016b plus C-terminus deletion (G292-L488)

Selected References:

- Dong J. et al., 2007, Cell, 130: 1120-1133
 Komuro, A., et al., 2003. J. Biol Chem 278. 33334-41
 Sudol K. et al., 1995. J Biol Chem 270. 14733-41
 Zhao B. et al., 2007. Genes Dev., 21: 2747-61

OMIM Link: <http://www.ncbi.nlm.nih.gov/omim/606608>