

# hJNKK1 ProtéGene™ Set

Catalog# J1106  
 Lot# Labeled on vial

## Materials Provided

1. pMEV2HA-JNKK1-WT (J1106a): 20µg in 40µl TE, 0.5mg/ml.
2. pMEV2HA-JNKK1-KM (J1106b): 20µg in 40 µl TE, 0.5mg/ml.
3. pMEV2HA-JNKK1-EE (J1106c): 20µg in 40µl TE, 0.5mg/ml.
4. pMEV2HA-JNKK1-AA (J1106d): 20µg in 40µl TE, 0.5mg/ml.
5. pMEV2HA-JNKK1-KM/AA (J1106e): 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, 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 human JNKK1 and mutants

Mitogen-activated protein kinases (MAPKs) cascade relays extracellular signals from cell membrane to the nucleus to induce intracellular responses and to regulate many aspects of cell physiology. These cascades, including JNK, ERK and p38 pathways, consist of distinct members of regulatory enzymes that serially activate one another in response to growth factors, cytokines and other mitogenic stimuli, leading to (in)activation of transcription factors. Like ERK1/2 and p38, the JNK activation requires dual phosphorylation on tyrosine and threonine residues within a conserved TPY motif. Activated JNKK1/2 can preferably phosphorylate and activate JNKs, with JNKK2 more specific to JNK. Please refer to the Selected References section for more information, or the Web Resources section for in depth references.

## Molecular Features of the inserts:

**Gene:** *Homo sapiens mitogen-activated protein kinase kinase 4 (MAP2K4)*, (Nickname JNKK1)  
**GenBank Reference Sequence:** NM\_003010  
**5'-Cloning Site:** Bam HI  
**5'-Junction Sequence:** 5'... tacctggtatcc ATG JNKK1...3'  
**3'-Cloning Site:** Kpn I  
**3'-Junction Sequence:** 5'... JNKK1 tagaggtacc TCA ...-3'

## Human JNKK1 Nucleotide and Protein Sequence

(1200 bps encoding 399 amino acid residues, with mutation locations underlined and marked in red )

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1  ATGGCGGCTC  CGAGCCCGAG  CGGCGGCGGC  GGCTCCGGGG  GCGGCAGCGG  CAGCGCCACC
   M A A P S P S G G G G G S G S G S G S G T
61  CCGCGCCCGG  TAGGTCCTCC  GCGCGCAGGC  CACCCGCGCG  TCAGCAGCAT  GCAGGGTAAA
   P G P V G S P A P G H P A V S S M Q G K
121 CGCAAAGCAC  TGAAGTTGAA  TTTTCAAAAT  CCACCTTCCA  AATCTACAGC  AAGGTTTACT
   R K A L K L N F A N P P F K S T A R F T
181 CTGATCCCA  ATCTACAGG  AGTTCAAAK  CCACACATAG  AGAGACTGAG  AACACACAGC
   L N P N P T G V Q N P H I E R L R T H S
241 ATTGAGTCAT  CAGAAAACCT  GAAGATCTCC  CCTGAACAC  ACTGGGATTT  CACTGCAGAG
   I E S S G K L K I S P E Q H W D F T A E
301 GACTTGAAG  ACCTTGGAGA  AATTGGACGA  GGAAGTTATG  GTTCTGTCAA  CAAATGTGC
   D L K D L G E I G R G A Y G S V N K M V
361 CACAAACCAA  GTGGCAAAT  AATGGCAGTT  AAAGAAATTC  GGTCAACAGT  GGATGAAAA
   H K P S G Q I M A V K R I R S T V D E K
421 GAACAAAAC  AACTTCTTAT  GGATTTGGAT  GTATGATGTC  GGAGTAGTGA  TTGCCATAC
   E Q K Q L L M D L D V V M R S S D C P Y
481 ATTGTTCACT  TTTATGGTGC  ACTTCTCAGA  GAGGGTACT  GTTGGATCTG  TGTGGTAACT
   I V Q F Y G A L F R E G D C W I C M E L
541 ATGTTACCT  CGTTTGATAA  GTTTTACAAA  TATGTATATA  GTGTATTAGA  TGATGTTATT
   M S T S F D K F Y K Y V Y S V L D D V I
601 CCAGAAGAAA  TTTTAGGCAA  AATCACTTTA  GCAACTGTGA  AAGCACTAAA  CCACTTAAAA
   P E E I L G K I T L A T V K A L N H L K
661 GAAAACCTGA  AAATTATCA  CAGAGATATC  AAACCTTCCA  ATATCTTCT  GGACAGAAGT
   F N L K I I H R D I K P S N I L L D R S
721 GGAATATTA  AGCTCTGTGA  CTTCGGCATC  ACTGGACAGC  TTGTGGACTC  TATGGCCAA
   G N I K L C D F G I S G Q L V D S I A K
781 ACAAGAGATG  CTGGCTGTAG  GCCATACATG  GCACCTGAAA  GAATGACCC  AAGCGCATCA
   T R D A G C R P Y M A P E R I D P S A S
841 CGACAAGGAT  ATGATGTCCG  CTCTGATGTC  TGGAGTTTGG  GGATCACATT  GTATGAGTTG
   R Q G Y D V R S D V W S L G I T L Y E L
901 GCCACAGGCC  GATTTCCCTA  TCCAAAGTGG  AATAGTGTAT  TTGATCACT  AACACAAGTC
   A T G R F P Y P K W N S V F D Q L T Q V
961 GTGAAAGGAG  ATCTCCGCA  GCTGAGTAAT  TCTGAGGAAA  GGGAAATCTC  CCGGATTTTC
   V K G D P P Q L S N S E E R E F S P S F
1021 ATCAACTTGT  TCAACTTGTG  CCTTACGAAG  KATGAATCCA  AAAGGCCAAA  GTATAAAGG
   I N F V N L C L T K D E S K R P K Y K E
1081 CTCTGGAAC  ATCCCTTAT  TTTGATGTAT  GAAGAAGCTG  CCGTGGAGGT  CCGATGCTAT
   L L K H P F I L M Y E E R A V E V A C Y
1141 GTTTGTAAAA  TCTGGATCA  AATGCCAGCT  ACTCCAGCT  CTCCATGTA  TGTGATTTGA
   V C K I L D Q M P A T P S S P M Y V D -
  
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## Mutations:

pMEV-JNKK1-WT (J1106a): No mutation  
 pMEV-JNKK1-K131M (J1106b): K131M: AAA→ATG  
 pMEV-JNKK1-EE (J1106c): S257E: TCT→GAG, T261E: ACA→GAA  
 pMEV-JNKK1-AA (J1106d): S257A: TCT→GCT, T261A: ACA→GCA  
 pMEV-JNKK1-KM/AA (J1106e): K131M: AAA→ATG, S257A: TCT→GCT, T261A: ACA→GCA

## Selected References:

- Lin A., et al, Identification of a dual specificity kinase that activates the Jun kinases and p38-Mpk2. *Science* 268: 286-290, 1995.  
 PubMed ID : 7716521
- Roux PP, Blenis J, ERK and p38 MAPK-activated protein kinases: a family of protein kinases with diverse biological functions. *Microbiol Mol Biol Rev* 68(2):320-344, 2004
- Kyriakis JM and Avruch J, Mammalian mitogen-activated protein kinase signal transduction pathways activated by stress and inflammation. *Physiol. Rev.* 81(2) 807-869, 2001

## Web Resources:

For sequence, references and a comprehensive description, please click the links below or copy and paste the link to your browser address bar:  
<http://www.ncbi.nlm.nih.gov/entrez/viewer.fcgi?db=nucleotide&val=24497520>  
<http://www.ncbi.nlm.nih.gov/entrez/dispomim.cgi?id=601335>