

hJNKK2 ProtéGene™ Set

Catalog# J1108
 Lot# Labeled on vial

Materials Provided

1. pMEV2HA-JNKK2-WT (J1108a): 20µg in 40µl TE, 0.5mg/ml.
2. pMEV2HA-JNKK2-KM (J1108b): 20µg in 40 µl TE, 0.5mg/ml.
3. pMEV2HA-JNKK2-EE (J1108c): 20µg in 40µl TE, 0.5mg/ml.
4. pMEV2HA-JNKK2-AA (J1108d): 20µg in 40µl TE, 0.5mg/ml.
5. pMEV2HA-JNKK2-KM/AA (J1108e): 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 JNKK2 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 7 (MAP2K7)*, (Nickname JNKK2)

GenBank Reference Sequence: NM_145185

5'-Cloning Site: Bam HI

5'-Junction Sequence: 5'... ggtacc ATG JNKK2...3'

3'-Cloning Site: Kpn I

3'-Junction Sequence: 5'... JNKK2 ggtacc TGA ...3'

Human JNKK2 Nucleotide and Protein Sequence

(1206 bps encoding 401 amino acid residues, with mutation locations underlined and marked in red)

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1 ATGGCGGCGT CCTCCCTGGA ACAGAAGCTG TCCCGCGCTG AAGCAAAGCT GAAGCAGGAG
  M A A S S L E Q K L S R L E A K L K Q E
61 AACCGGGAGG CCGCGGGAGG GATCGACCTC AACCTGGATA TCAGCCCCCA GCGGCCCAGG
  N R E A R R R I D L N L D I S F Q R P R
121 CCCACCTGCG AGCTCCCGCT GGCCCAAGAT GGGGCGCAGC GCTCCGCATC CTCAGAGAGC
  P T L Q L P L A N D G G S R S P S S E S
181 TCCCGCGCAG ACCCCAGCCG CCCCAGCCCG CCCCAGCACA TGCTGGGGCT CCGTCAACCC
  S P Q H P T P P A R P R H M L G L P S T
241 CTGTTCCACG CCGCGAGCAT GGAGGACMTT GAGATTGACC ACAAGCTGCA GGAGATCATG
  L F T P R S M E S I E I D H K L Q E I M
301 AAGCAGACGG GCTACTGTAC CATCGGGGCG CAGCGCTACC AGGCGAAT CAACGACCTG
  K Q T G Y L T I G G Q R Y Q A E I N D L
361 GAGAAGTTGG GCGAGATGGG CAGCGGCACC TCGCGACCGG TGTGGAAGAT GCGATTCCGG
  E N L G E M G S G T C G P V W K M R F R
421 AAGACCGGCG ACGTCAATGC CGTTAAGCAA ATGCGGCGAT CGGGGAACAA GGAGGAGAAC
  K T G H V I A V K C M M R R S G N K E E N
481 AAGCGCATCC TCATGGACCT GGATGTGGTG CTGAAGACCC ACGACTGCCC CTACATCGTG
  K R I L M D L D V V L K S H D C P Y I V
541 CAGTGGCTTG GGACGTTTCAT CACCAACACG GACGTTCTCA TCGCCATGGA GCTCATGGGG
  Q C F G T F I T N T D V F I A M E L M G
601 ACCTGCGCTG AGAAGCTCAA GAAGCGGATG CAGGCGCCCA TCCCGAGGCG CATTTCTGGG
  T C A E K L K K R M Q G P I P E R I L G
661 AAGATGACAG TGGCGATTGT GAGGCGCTGT TACTACTGTA AGGAGAAGCA CGGTGTCATC
  K M T V A I V K A L Y Y L K E K H G V I
721 CACCGCGAGC TCAAGCCCTC CAACATCTGT CTGAGCAGAG GGGGCGAGAT CAAGCTCTGC
  H R D V K P S N I L L D E R G Q I K L C
781 GACTTCGGCA TCAGCGGCGG CCTGGTGGAC TCCAAAGCCA AGACCGGAG CCGCGGCTGT
  D F G I S G R L V D S K A K I R S A G C
841 GCGCGCTACA TGGCACCCGA GCGCATTGAC CCCCAGACCC CCACCAAGCC GGACTATGAC
  A A Y M A P E R I D P P D P T K P D Y D
901 ATCCGGGCGG ACGTATGGAG CCGTGGGATG TCGTTGGTGG AGCTGGCAAC AGGACAGTTT
  I R A D V W S L G I S L V E L A T G Q F
961 CCCTACAAGA ACTGCAAGAC GGACTTTGAG GTCTCAACCA AAGTCTTACA GGAAGAGCCC
  P Y K N C K T D F E V L T K V L Q E E P
1021 CCGCTTCTGC CCGGACACAT GGGCTTCTCG GGGGACTTCC AGTCCTTCTG CAAAGACTGC
  P L L P G H M G F S G D F Q S F V K D C
1081 CTTACTAAG ATCACAGGAA GAGACCAAAG TATAATAAGC TACTTGAACA CAGTTCATC
  L T K D H R K R P K Y N K L L E H S F I
1141 AAGCGCTACG AGACGCTGGA GGTGGACGTG GCGTCTGGT TCAAGGATGT CATGGCGAAG
  K R Y E T L E V D V A S W F K D V M A K
1201 ACCTGA
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Mutations:

pMEV-JNKK2-WT (J1108a): No mutation
 pMEV-JNKK2-K131M (J1108b): K149M: AAG→ATG
 pMEV-JNKK2-EE (J1108c): S271E: TCC→GAG, T275E: ACG→GAA
 pMEV-JNKK2-AA (J1108d): S271A: TCC→GCT, T275A: ACG→GCA
 pMEV-JNKK2-KM/AA (J1108d): K131M: AAA→ATG, S271A: TCC→GCT, T275A: ACG→GCA

Selected References:

- Lu X, Nemoto S and Lin A, Identification of c-jun NH(2)-terminal protein kinase (JNK)-activating kinase 2 as an activator of JNK but not p38. *J Biol Chem* 272:24751-24754, 1997
- Wu Z et al, Molecular cloning and characterization of human JNKK2, a novel jun NH(2)-terminal kinase-specific kinase. *Molec Cell Biol* 17:7407-7416, 1997
- 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/dispmim.cgi?id=603014>