

hJNKK2 ProtéGene™ Set

Catalog# J1108
Lot# Labeled on vial

Materials Provided

1. pMEV2HA-JNKK2-WT (J1108a): 20 μ g in 40 μ l TE, 0.5mg/ml.
2. pMEV2HA-JNKK2-K149M (J1108b): 20 μ g in 40 μ l TE, 0.5mg/ml.
3. pMEV2HA-JNKK2-EE (J1108c): 20 μ g in 40 μ l TE, 0.5mg/ml.
4. pMEV2HA-JNKK2-AA (J1108d): 20 μ g in 40 μ l TE, 0.5mg/ml.
5. pMEV2HA-JNKK2-KMAA (J1108e): 20 μ g in 40 μ l TE, 0.5mg/ml.
6. Product Information Sheet.

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

Receiving and Storage:

If received in lyophilized form, add 40 μ l 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 25-50 μ g/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'... ggtatc 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 marked in red & underlined.)

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1  ATGGCGGGCT CCTCCCTGGA ACAGAAAGCTG TCCCGCTTGG AAGCAAGCT GAAGCAGGAG
61 AACCGGGAGG CCCGGCGGAG GATCGACCTTC AACCTGTATA TCAGCCCCCA CGGGCCCCAGG
N R E R R I D L N L D I S P R P R
121 CCCCGCTTCG AGCTCCCGGT GGCGCACCGT GGGGGCAGCC GCTGCCCATC CTAGAGAGC
P T L Q L P L A N D G G S R S P S S E S
181 TCCCCGGAGC ACCCCCCAGCC CCCCCCGGGG CCCCCGCACA TCCCTGGGCT CCCCTCAACC
S P Q H P T P P R P R H M L G L P S T
241 CTGTTACACAC CCUCGAGCATC GGAGAGCATT GAGATTGACCA ACAAGCTGCA GGAGATCATG
L F T P R S M E S I E I D H K L Q E I M
301 AAGCCGAGCCG GCTACCTGAC CATCGGGGGC CAGCGCTTACCC AGGCAGAAAT CAACGGACCTG
K Q T G Y L T I G G Q R Y Q A E I N D L
361 GAGAACCTTGG CGCAGATGGG CAGCGACCC TGCGGACCCGG TGTGGAAGAT CGCGTTCCGG
E N L G E M G S T C G P V W K M R F R
421 AAAGACGGGCC AGCTGATTGCG CGTTAACCAA ATCGGGCGAT CGGGGAGACAA GGAGGGAGAC
K T G H V I A V E K Q M R R S G N K E E N
481 AAAGCGATTC TCATGACCTT GGATGTGGTG CTGAAGACCC AGCAGCTGGCC CTACATGCTG
K R I L M D L D V V L K S H D C P Y I V
541 CAGTGGTTTG GCGAGCTTACG CACCAACCCG GAGCTCTTCA TGCGCATGGG GCTCATGGC
Q C P G T F I T N T D V F I A M E L M G
601 ACCTGGCTG AGAAAGCTCAA AAAGCGGATG CAGGGCCCA TCCCCGAGCG CATTCTGGC
T C A E K L K K R M Q G P I P E R I L G
661 AAAGATGACAG TGGCGATTGTG AAAGCGCTG TACTACTTGA AGGAGAGCA CGGTGTGATC
K M T A I V K A L Y L K E K H G V I
721 CACCGGGAGC TCAAGCCCTC CAACATCTTG CTGGAGAGC GGGGGAGAT CAACGCTTCG
H R D V K P S N I L L D E R G Q I K L C
781 GACTTCGGCA TCAGCGGGCG CCTGGTGGAC TCCAAAGCCAG AGACGGGAG CGCCGGCTGT
D F G I S G R L V D S K A K T R S A G C
841 GCGCCCTACA TGCGCACCGGA GGCGCATTTGAC CCCCCAGACCC CACCAAGCC GGACATATGAC
A A Y M A P E R I D P P D P T K P D Y D
901 ATCCGGGGC AGCTGATGGG CCTGGGCGATC TTGGTGTGGG AGCTGGCAC AGGGACAGTT
I R A D V W S L G I S L V E L A T G Q F
961 CCCTACAGA ACTCGAACAG GGAATGTGGAG GTCTCACCA AAGTCTTACA GGAAGAGCCC
P Y K N C K T D F E V L T K V L Q E E P
1021 CGCGCTTCGCG CGGACACAT GGCGCTTCG GGGGACTTCC AGTCCCTCGT CAAAGACTC
P L L P G H M G F S G D F Q S F V K D C
1081 CCTTACTAAAG ATCACAGGA GAGACCAAG TATAATAAGC TACTTGAACA CAGCTGATC
L T K D H R K P R V Y N K L L E H S F I
1141 AAAGCGCTACG AGACGCTGGA GGTGGACGTG GCCTCTGGT TCAAGGATGT CATGGCAAG
K R Y E T L E V D V A S W F K D V M A K
1201 ACCTGA T -

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Mutations:

JNKK2-WT (J1108a):	No mutation
JNKK2-K149M (J1108b):	K149M: AAG→ATG
JNKK2-EE (J1108c):	S271E: TCC>GAG, T275E: ACG→GAG
JNKK2-AA (J1108d):	S271A: TCC→GCC, T275A: ACG→GCG
JNKK2-KMAA(J1108e):	K149M: AAG → ATG S271A: TCC→GCC T275A: ACG→GCG

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>