Technology
Biomyx
Innovation & Convenience for Life Sciences

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JNK1 ProteGene™ Set

Catalog# J1105
Lot# Labeled on vial

Materials Provided

1. pMEV2HA-JNK1-WT (J1105a): 20 μg in 40 μl TE, 0.5 mg/ml.
2. pMEV2HA-JNK1-K55M (J1105b): 20 μg in 40 μl TE, 0.5 mg/ml.
3. pMEV2HA-JNK1-EE (J1105c): 20 μg in 40 μl TE, 0.5 mg/ml.
4. pMEV2HA-JNK1-AA (J1105d): 20 μg in 40 μl TE, 0.5 mg/ml.
5. Product Information Sheets.

Note: *Individual plasmids can be ordered separately. Some plasmids are shipped as lyophilized pellet.*

Receiving and Storage:

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. 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 and 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. #LK-1100, Prepured 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 JNK1 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. Activation by external stimuli like UV of the Jun N-terminal Kinase (JNK) pathway increases the expression of a set of autoimmune and inflammatory genes, including IL-2 and gamma interferon. Multiple subtypes of the enzyme induce cell- and stimulus- specific responses. The mutation K55M renders the enzyme catalytically inactive.

Molecular Features of the inserts:

Gene: *Homo sapiens* mitogen-activated protein kinase 8 (MAPK8), transcript variant 2
GenBank Reference Sequence: NM_002750
5'-Cloning Site: Bam HI
5'-Junction Sequence: 5'-…tac gct gga tcc **ATG AGC AGA**-…3'
3'-Cloning Site: Kpn I
3'-Junction Sequence: 5'-…actctagaggtacc **TCA** TCT ACA …-3'

hJNK1 Nucleotide and Protein Sequence

(1155 bps endoding 384 amino acid residues. Nucleotides encoding K55, T183 and Y185 are in red and underlined)

```
   1  atgagcagaa gcaagcgtga caacaatttt tatagtgtag agattggaga ttctacattc
        M  S  R   S  K  R   D  N  N  F   Y  S  V   E  I  G   D  S  T  F
  61  acagtcctga aacgatatca gaatttaaaa cctataggct caggagctca aggaatagta
        T  V  L   K  R  Y   Q  N  L  K   P  I  G   S  G  A   Q  G  I  V
 121  tgcgcagctt atgatgccat tcttgaaaga aatgttgcaa tcAAGaagct aagccgacca
        C  A  A   Y  D  A   I  L  E  R   N  V  A   I  K  K   L  S  R  P
 181  tttcagaatc agactcatgc caagcgggcc tacagagagc tagttcttat gaaatgtgtt
        F  Q  N   Q  T  H   A  K  R  A   Y  R  E   L  V  L   M  K  C  V
 241  aatcacaaaa atataattgg ccttttgaat gttttcacac cacagaaatc cctagaagaa
        N  H  K   N  I  I   G  L  L  N   V  F  T   P  Q  K   S  L  E  E
 301  tttcaagatg tttacatagt catggagctc atggatgcaa atctttgcca agtgattcag
        F  Q  D   V  Y  I   V  M  E  L   M  D  A   N  L  C   Q  V  I  Q
 361  atggagctag atcatgaaag aatgtcctac cttctctatc agatgctgtg tggaatcaag
        M  E  L   D  H  E   R  M  S  Y   L  L  Y   Q  M  L   C  G  I  K
 421  caccttcatt ctgctggaat tattcatcgg gacttaaagc ccagtaatat agtagtaaaa
        H  L  H   S  A  G   I  I  H  R   D  L  K   P  S  N   I  V  V  K
 481  tctgattgca ctttgaagat tcttgacttc ggtctggcca ggactgcagg aacgagtttt
        S  D  C   T  L  K   I  L  D  F   G  L  A   R  T  A   G  T  S  F
 541  atgatgACGc ctTATgtagt gactcgctac tacagagcac ccgaggtcat ccttggcatg
        M  M  T   P  Y  V   V  T  R  Y   Y  R  A   P  E  V   I  L  G  M
 601  ggctacaagg aaaacgtgga tttatggtct gtggggtgca ttatgggaga aatggtttgc
        G  Y  K   E  N  V   D  L  W  S   V  G  C   I  M  G   E  M  V  C
 661  cacaaaatcc tctttccagg aagggactat attgatcagt ggaataaagt tattgaacag
        H  K  I   L  F  P   G  R  D  Y   I  D  Q   W  N  K   V  I  E  Q
 721  cttggaacac catgtcctga attcatgaag aaactgcaac caacagtaag gacttacgtt
        L  G  T   P  C  P   E  F  M  K   K  L  Q   P  T  V   R  T  Y  V
 781  gaaaacagac ctaaatatgc tggatatagc tttgagaaac tcttccctga tgtccttttc
        E  N  R   P  K  Y   A  G  Y  S   F  E  K   L  F  P   D  V  L  F
 841  ccagctgact cagaacacaa caaacttaaa gccagtcagg caagggattt gttatccaaa
        P  A  D   S  E  H   N  K  L  K   A  S  Q   A  R  D   L  L  S  K
 901  atgctggtaa tagatgcatc taaaaggatc tctgtagatg aagctctcca acacccgtac
        M  L  V   I  D  A   S  K  R  I   S  V  D   E  A  L   Q  H  P  Y
 961  atcaatgtct ggtatgatcc ttctgaagca gaagctccac caccaaagat ccctgacaag
        I  N  V   W  Y  D   P  S  E  A   E  A  P   P  P  K   I  P  D  K
1021  cagttagatg aaagggaaca cacaatagaa gagtggaaag aattgatata taaggaagtt
        Q  L  D   E  R  E   H  T  I  E   E  W  K   E  L  I   Y  K  E  V
1081  atggacttgg aggagagaac caagaatgga gttatacggg ggcagccctc tcctttagca
        M  D  L   E  E  R   T  K  N  G   V  I  R   G  Q  P   S  P  L  A
1141  caggtgcagc agtga
        Q  V  Q   Q  -
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Mutations:

pMEV-JNK1-WT (J1105a): No mutation
pMEV-JNK1-K55M (J1105b): K55M: AAG→ATG
pMEV2HA-JNK1-EE (J1105c): T183E: ACG →GAG
Y185E: TAT→GAA
pMEV2HA-JNK1-AA (J1105d): T183A: ACG →GCG
Y185A: TAT→GCT

Selected References:

Derijard B, et al, JNK1: a protein kinase stimulated by UV light and Ha-Ras that binds and phosphorylates the c-Jun activation domain. Cell 76(6):1025-1037, 1994

Nakano H. Signaling crosstalk between NF-kappaB and JNK. Trends Immunol 25(8):402-405, 2004

Web Resources:

http://www.ncbi.nlm.nih.gov/entrez/viewer.fcgi?db=nucleotide&val=20986493
http://www.ncbi.nlm.nih.gov/entrez/dispomim.cgi?id=601158