Technology
Biomyx
Innovation & Convenience for Life Sciences

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P38 ProtéGene™ Set

Catalog# P1070
Lot# Labeled on vial

Materials Provided

1. pMEV2HA-P38-WT (P1070a): 20 μg in 40 μl TE, 0.5 mg/ml.
2. pMEV2HA-P38-K53M (P1070b): 20 μg in 40 μl TE, 0.5 mg/ml.
3. pMEV2HA-P38-EE (P1070c): 20 μg in 40 μl TE, 0.5 mg/ml.
4. pMEV2HA-P38-AA (P1070d): 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 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^{0}C if used immediately or, 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.cat#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 P38 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. Proinflammatory cytokine and microbial products activate p38 (SAPK2) gene regulating pathway and cause the expression of multiple cytokine genes including IL-1, IL-6 and TNF alpha. The mutation p38 K53M renders the enzyme catalytically inactivate.

Molecular Features of the inserts:

Gene: *Homo sapiens* mitogen-activated protein kinase 14 (MAPK14), transcript variant 1
GenBank Reference Sequence: NM_001315
5'-Cloning Site: Bam HI
5'-Junction Sequence: 5'-…tacgctggatcc **ATG TCT CAG**-…3'
3'-Cloning Site: Kpn I
3'-Junction Sequence: 5'-…ctctagaggtacc **TCA GGA CTC** …-3'

hP38 Nucleotide and Protein Sequence

(1083 bps endoding 360 amino acid residues. Nucleotides encoding K53, T180 and Y183 are in bold and underlined)

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   1  atgtctcagg agaggcccac gttctaccgg caggagctga acaagacaat ctgggaggtg
        M  S  Q   E  R  P   T  F  Y  R   Q  E  L   N  K  T   I  W  E  V
  61  cccgagcgtt accagaacct gtctccagtg ggctctggcg cctatggctc tgtgtgtgct
        P  E  R   Y  Q  N   L  S  P  V   G  S  G   A  Y  G   S  V  C  A
 121  gcttttgaca caaaaacggg gttacgtgtg gcagtgAAGa agctctccag accatttcag
        A  F  D   T  K  T   G  L  R  V   A  V  K   K  L  S   R  P  F  Q
 181  tccatcattc atgcgaaaag aacctacaga gaactgcggt tacttaaaca tatgaaacat
        S  I  I   H  A  K   R  T  Y  R   E  L  R   L  L  K   H  M  K  H
 241  gaaaatgtga ttggtctgtt ggacgttttt acacctgcaa ggtctctgga ggaattcaat
        E  N  V   I  G  L   L  D  V  F   T  P  A   R  S  L   E  E  F  N
 301  gatgtgtatc tggtgaccca tctcatgggg gcagatctga acaacattgt gaaatgtcag
        D  V  Y   L  V  T   H  L  M  G   A  D  L   N  N  I   V  K  C  Q
 361  aagcttacag atgaccatgt tcagttcctt atctaccaaa ttctccgagg tctaaagtat
        K  L  T   D  D  H   V  Q  F  L   I  Y  Q   I  L  R   G  L  K  Y
 421  atacattcag ctgacataat tcacagggac ctaaaaccta gtaatctagc tgtgaatgaa
        I  H  S   A  D  I   I  H  R  D   L  K  P   S  N  L   A  V  N  E
 481  gactgtgagc tgaagattct ggattttgga ctggctcggc acacagatga tgaaatgACA
        D  C  E   L  K  I   L  D  F  G   L  A  R   H  T  D   D  E  M  T
 541  ggcTACgtgg ccactaggtg gtacagggct cctgagatca tgctgaactg gatgcattac
        G  Y  V   A  T  R   W  Y  R  A   P  E  I   M  L  N   W  M  H  Y
 601  aaccagacag ttgatatttg gtcagtggga tgcataatgg ccgagctgtt gactggaaga
        N  Q  T   V  D  I   W  S  V  G   C  I  M   A  E  L   L  T  G  R
 661  acattgtttc ctggtacaga ccatattaac cagcttcagc agattatgcg tctgacaggg
        T  L  F   P  G  T   D  H  I  N   Q  L  Q   Q  I  M   R  L  T  G
 721  acacccccg cttatctcat taacaggatg ccaagccatg aggcaagaaa ctatattcag
        T  P  P   A  Y  L   I  N  R  M   P  S  H   E  A  R   N  Y  I  Q
 781  tctttgactc agatgccgaa gatgaacttt gcgaatgtat ttattggtgc caatcccctg
        S  L  T   Q  M  P   K  M  N  F   A  N  V   F  I  G   A  N  P  L
 841  gctgtcgact tgctggagaa gatgcttgta ttggactcag ataagagaat tacagcggcc
        A  V  D   L  L  E   K  M  L  V   L  D  S   D  K  R   I  T  A  A
 901  caagcccttg cacatgccta ctttgctcag taccacgatc ctgatgatga accagtggcc
        Q  A  L   A  H  A   Y  F  A  Q   Y  H  D   P  D  D   E  P  V  A
 961  gatccttatg atcagtcctt tgaaagcagg gacctcctta tagatgagtg gaaaagcctg
        D  P  Y   D  Q  S   F  E  S  R   D  L  L   I  D  E   W  K  S  L
1021  acctatgatg aagtcatcag ctttgtgcca ccacccattg accaagaaga gatggagtcc
        T  Y  D   E  V  I   S  F  V  P   P  P  I   D  Q  E   E  M  E  S
1081  tga
        -
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Mutations:

pMEV-P38-WT (P1070a): No mutation
pMEV-P38-K53M (P1070b): K53M: AAG ⟶ ATG
pMEV2HA-P38-EE (P1070c): T183E: ACA →GAA
Y185E: TAC→GAA
pMEV2HA-P38-AA (P1070d): T183A: ACA →GCA
Y185A: TAC→GCC

Selected References:

Han J, et al, Molecular cloning of human p38 MAP kinase. Biochim Biophys Acta 1265(2-3):224-227, 1995
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
Saccani S, Pantano S and Natoli G, p38-dependent marking of inflammatory genes for increased NF-B recruitment. Nature Immunol 3:69-75, 2002

Web Resources:

http://www.ncbi.nlm.nih.gov/entrez/viewer.fcgi?db=nucleotide&val=4503068
http://www.ncbi.nlm.nih.gov/entrez/dispomim.cgi?id=600289