Technology Biomyx
Innovation & Convenience for Life Sciences

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MST2 ProteGene™ Set

Catalog# H1012
Lot# 140071

Materials Provided

1. pMEV2HA-MST2-WT (H1012a): 20 μg in 40 μl TE (0.5 mg/ml).
2. pMEV2HA-MST2-K56R (H1012b):20 μg in 40 μl TE(0.5 mg/ml).
3. Product Information Sheets.

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

Receiving and Storage:

If received in liquid form, spin the vials briefly in a microcentrifuge to collect the contents. 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. 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 MST2 and Mutants

MST2 is a serine/threonine kinase originally identified from a HeLa cell (Creasy and Chernoff, 1995) and Jurkat (Taylor et al., 1996) cDNA libraries. Yeast 'sterile 20' (Ste20) kinase is MST2's homolog and acts upstream of the mitogen-activated protein kinase (MAPK) cascade that is activated under a variety of stress conditions. In mammalian cells, MST2 activated by the proapoptotic agents straurosporine and FAS ligand (Taylor et al., 1996; Lee et al., 2001). Gene knock out experiments indicated that MST2 activation is associated with apoptosis (O'Neil et al., 2004). MST2, and it's homolog in Drosophila (hippo, Hpo kinase), is a part of the Hippo kinase cascade that was recently shown to promote cell proliferation and inhibits apoptosis (Zhao et al., 2008; Dong et al., 2007, Harvey et al., 2003).

MST2 protein contains 491-amino acid and can phosphorylate myelin basic protein (MBP), histone H1, and itself. The catalytic domain of MST2 is at the N-terminus of the protein. Lysine residue 56 is a catalytically essential residue, and hence, mutating it to arginine abolishes the kinase activity of MST2. Therefore, the K56R-expressing vector (H1012b) can be used as a dominant negative to inhibit Hippo pathway in mammalian cells.

Molecular Features of the inserts:

Gene: *Homo sapiens* Serine/Threonine Kinase 3 (STK3)
GenBank Reference Sequence: NM_006281
Synonyms: MST-2, mammalian serine/threonine kinase 3 (STK3); STE20-like kinase MST2; mammalian STE20-like protein kinase 2; serine/threonine-protein kinase Krs-1;
5'-Cloning Site: Bam HI
5'-Junction Sequence(upper strand) :
5'-…tac gct gga tcc **ATG GAG CAG**…3'
3'-Cloning Site: Xba I
3'-Junction Sequence (lower strand):
5'-…gac tct aga **TCA AAA GTT TTG** …-3'

MST2 Nucleotide & Amino Acid Sequences

(1476 bps. Codon for K56 is in bold and underlined)

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   1  atggagcagc cgccggcgcc taagagtaaa ctaaaaaagc tgagtgaaga cagtttgact
        M  E  Q   P  P  A   P  K  S  K   L  K  K   L  S  E   D  S  L  T
  61  aagcagcctg aagaagtttt tgatgtatta gagaagcttg gagaagggtc ttatggaagt
        K  Q  P   E  E  V   F  D  V  L   E  K  L   G  E  G   S  Y  G  S
 121  gtatttaaag caatacacaa ggaatccggt caagttgtcg caattAAAca agtacctgtt
        V  F  K   A  I  H   K  E  S  G   Q  V  V   A  I  K   Q  V  P  V
 181  gaatcagatc ttcaggaaat aatcaaagaa atttccataa tgcagcaatg tgacagccca
        E  S  D   L  Q  E   I  I  K  E   I  S  I   M  Q  Q   C  D  S  P
 241  tatgttgtaa agtactatgg cagttatttt aagaatacag acctctggat tgttatggag
        Y  V  V   K  Y  Y   G  S  Y  F   K  N  T   D  L  W   I  V  M  E
 301  tactgtggcg ctggctctgt ctcagacata attagattac gaaacaagac attaatagaa
        Y  C  G   A  G  S   V  S  D  I   I  R  L   R  N  K   T  L  I  E
 361  gatgaaattg caaccattct taaatctaca ttgaaaggac tagaatattt gcactttatg
        D  E  I   A  T  I   L  K  S  T   L  K  G   L  E  Y   L  H  F  M
 421  agaaaaatac acagagatat aaaagctgga aatattctcc tcaatacaga aggacatgca
        R  K  I   H  R  D   I  K  A  G   N  I  L   L  N  T   E  G  H  A
 481  aaattggcag attttggagt ggctggtcag ttaacagata caatggcaaa acgcaatact
        K  L  A   D  F  G   V  A  G  Q   L  T  D   T  M  A   K  R  N  T
 541  gtaataggaa ctccattttg gatggctcct gaggtgattc aagaaatagg ctataactgt
        V  I  G   T  P  F   W  M  A  P   E  V  I   Q  E  I   G  Y  N  C
 601  gtggccgaca tctggtccct tggcattact tctatagaaa tggctgaagg aaaacctcct
        V  A  D   I  W  S   L  G  I  T   S  I  E   M  A  E   G  K  P  P
 661  tatgctgata tacatccaat gagggctatt tttatgattc ccacaaatcc accaccaaca
        Y  A  D   I  H  P   M  R  A  I   F  M  I   P  T  N   P  P  P  T
 721  ttcagaaagc cagaactttg gtccgatgat ttcaccgatt ttgttaaaaa gtgtttggtg
        F  R  K   P  E  L   W  S  D  D   F  T  D   F  V  K   K  C  L  V
 781  aagaatcctg agcagagagc tactgcaaca caacttttac agcatccttt tatcaagaat
        K  N  P   E  Q  R   A  T  A  T   Q  L  L   Q  H  P   F  I  K  N
 841  gccaaacctg tatcaatatt aagagacctg atcacagaag ctatggagat caaagctaaa
        A  K  P   V  S  I   L  R  D  L   I  T  E   A  M  E   I  K  A  K
 901  agacatgagg aacagcaacg agaattggaa gaggaagaag aaaattcgga tgaagatgag
        R  H  E   E  Q  Q   R  E  L  E   E  E  E   E  N  S   D  E  D  E
 961  ctggattccc acaccatggt gaagactagt gtggagagtg tgggcaccat gcgggccaca
        L  D  S   H  T  M   V  K  T  S   V  E  S   V  G  T   M  R  A  T
1021  agcacgatga gtgaaggggc ccagaccatg attgaacata atagcacgat gttggaatcc
        S  T  M   S  E  G   A  Q  T  M   I  E  H   N  S  T   M  L  E  S
1081  gacttgggga ccatggtgat aaacagtgag gatgaggaag aagaagatgg aactatgaaa
        D  L  G   T  M  V   I  N  S  E   D  E  E   E  E  D   G  T  M  K
1141  agaaatgcaa cctcaccaca agtacaaaga ccatctttca tggactactt tgataagcaa
        R  N  A   T  S  P   Q  V  Q  R   P  S  F   M  D  Y   F  D  K  Q
1201  gacttcaaga ataagagtca cgaaaactgt aatcagaaca tgcatgaacc cttccctatg
        D  F  K   N  K  S   H  E  N  C   N  Q  N   M  H  E   P  F  P  M
1261  tccaaaaacg tttttcctga taactggaaa gttcctcaag atggagactt tgacttttg
        S  K  N   V  F  P   D  N  W  K   V  P  Q   D  G  D   F  D  F  L
1321  aaaaatctaa gtttagaaga actacagatg cggttaaaag cactggaccc catgatggaa
        K  N  L   S  L  E   E  L  Q  M   R  L  K   A  L  D   P  M  M  E
1381  cgggagatag aagaacttcg tcagagatac actgcgaaaa gacagcccat tctggatgcg
        R  E  I   E  E  L   R  Q  R  Y   T  A  K   R  Q  P   I  L  D  A
1441  atggatgcaa agaaaagaag gcagcaaaac ttttga
        M  D  A   K  K  R   R  Q  Q  N   F  -
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Mutations:

pMEV-MST2-WT (H1012a): No mutation
pMEV-MST2-K56R(H1012b): K56R (AAA to AGA)

Selected References:

Creasy, C. L., et al., 1995. Gene 167: 303-306
Dong J. et al., 2007, Cell, 130: 1120-1133
Harvey, K. F., et al., 2003. Cell 114: 457-467
Lee, K.-K., et al., 2001. J. Biol. Chem. 276: 19276-19285
O'Neil E. et al., 2004. Science 306: 2267-2270
Taylor, L. K., et al., 1996. Proc. Nat. Acad. Sci. 93: 10099-104,
Zhao B. et al., 2007. Genes Dev., 21: 2747-61

OMIM Link: http://www.ncbi.nlm.nih.gov/omim/605030