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MEKK1 ProtéGene™ Set

Catalog# P1045
Lot# 283081

Materials Provided:

1. pMEVHA-MEKK1-KD (P1045a): 20 μg in 40 μl TE (pH7.5), 0.5 mg/ml.
2. pMEVHA-MEKK1-K452M (P1045b): 20 μg in 40 μl TE (pH7.5), 0.5 mg/ml.
3. 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.

Expression Vector:

pMEV-2HA (a): Cat# P1001a.

Affinity Tag:

N-terminal 2 x HA, a 9-aa peptide derived from influenza virus (MGYPYDVPDYAYPYDVPDYAGS…) .

Prokaryotic Selection:

The kanamycin-resistance gene (aminoglcoside 3' phospho-transferase) expression cassette in the plasmids confers Kanamycin resistance to bacteria cells. Bacterial cells (e.g. DH5α, JM109 or XL10-Gold) transformed with the plasmids should be maintained and grown in media containing 25-50μg/ml Kanamycin (e.g. Cat# LK-1100, Prepoured 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.

About MEKK1 And Mutants

MAPK pathways play a key role in mediating cell growth and apoptosis. Among the components of the MAPK family, mitogen-activated protein kinase kinase kinase (MEKK1) is a 196-kDa serine-threonine kinase that was first cloned from a cDNA library derived from NIH 3T3 cells by virtue of its homology to Ste11 and Byr2 (5). MEKK1 is activated in response to cytokines and various stresses, with GSK3beta as one of the direct activators (4). MEKK1 preferentially activates the JNK pathway and also influences the activity of the ERK pathway, while it has little or no effect on the p38-MAPK pathways. MEKK1 has been reported to be involved in cardiac hypertrophy (6, 8), mitochondrial permeability and apoptosis (2, 9), focal adhesion and cell migration (1, 10), activating C/EBP-beta-dependent gene expression in response to IFN-gamma (7).

MEKK1-KD is the c-terminal protein kinase domain of mouse MEKK1, consisting of amino acid residues 375-672. It is constitutively active and has been used to activate MAPK pathways in transfection assays (5, 11). Conserved in all protein kinases, lysine 452 in MEKK1 is catalytically essential. Changing it to a methionine, as is the case in MEKK1-K452M mutant, results in a mutant protein essentially lacking kinase activity.

Molecular Features of the Inserts:

Gene: *Mus musculus* MAPK/ERK Kinase Kinase 1 (MEKK1)
GenBank Entries: NM_011945; L13103; AF117340
5'-Cloning Site: Bam HI
5'-Junction Sequence: 5'-…tac gct gga tcc **ATG TCA GCG**-…3'
3'-Cloning Site: Eco RI
3'-Junction Sequence (lower strand):
5'-…acgcgt gaattc **CTA CCA CGT GGT**…-3'

mMEKK1-KD Amino Acid Sequence

(297 amino acid residues beginning with M375 in the full-length MEKK1 protein. K452 is in bold and underlined.)

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MSASQDALPIVPQLQVENGEDIIIIQQDTPETLPGHTKAKQPYREDAEWL
KGQQIGLGAFSSCYQAQDVGTGTLMAVKQVTYVRNTSSEQEEVVEALREE
IRMMGHLNHPNIIRMLGATCEKSNYNLFIEWMAGGSVAHLLSKYGAFKES
VVINYTEQLLRGLSYLHENQIIHRDVKGANLLIDSTGQRLRIADFGAAAR
LASKGTGAGEFQGQLLGTIAFMAPEVLRGQQYGRSCDVWSVGCAIIEMAC
AKPPWNAEKHSNHLALIFKIASATTAPSIPSHLSPGLRDVAVRCLELQPQ
DRPPSRELLKHPVFRTTW
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MEKK1-KD Nucleotide Sequence

(957bps beginning with nt1549 in entry L13103. Nucleotides encoding K452 is in bold and underlined)

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   1  ATGTCAGCGT CTCAGGATGC CCTCCCCATC GTCCCTCAGC TGCAGGTGGA
  51  AAATGGAGAA GATATTATCA TCATTCAGCA GGACACACCA GAAACTCTTC
 101  CAGGACATAC CAAAGCGAAA CAGCCTTACA GAGAAGACGC TGAGTGGCTG
 151  AAAGGCCAGC AGATAGGCCT CGGAGCATTT TCTTCCTGTT ACCAAGCACA
 201  GGATGTGGGG ACTGGGACTT TAATGGCTGT GAAACAGGTG ACGTACGTCA
 251  GAAACACATC CTCCGAGCAG GAGGAGGTGG TGGAAGCGTT GAGGGAAGAG
 301  ATCCGGATGA TGGGTCACCT CAACCATCCA AACATCATCC GGATGCTGGG
 351  GGCCACGTGC GAGAAGAGCA ACTACAACCT CTTCATTGAG TGGATGGCGG
 401  GAGGATCTGT GGCTCACCTC TTGAGTAAAT ACGGAGCTTT CAAGGAGTCA
 451  GTCGTCATTA ACTACACTGA GCAGTTACTG CGTGGCCTTT CCTATCTCCA
 501  CGAGAACCAG ATCATTCACA GAGACGTCAA AGGTGCCAAC CTGCTCATTG
 551  ACAGCACCGG TCAGAGGCTG AGAATTGCAG ACTTTGGAGC TGCTGCCAGG
 601  TTGGCATCAA AAGGAACCGG TGCAGGAGAG TTCCAGGGAC AGTTACTGGG
 651  GACAATTGCA TTCATGGCGC CTGAGGTCCT AAGAGGTCAG CAGTATGGTA
 701  GGAGCTGTGA TGTATGGAGT GTTGGCTGCG CCATTATAGA AATGGCTTGT
 751  GCAAAACCAC CTTGGAATGC AGAAAAACAC TCCAATCATC TCGCCTTGAT
 801  ATTTAAGATT GCTAGCGCAA CTACTGCACC GTCCATCCCG TCACACCTGT
 851  CCCCGGGTCT GCGCGACGTG GCCGTGCGCT GCTTAGAACT TCAGCCTCAG
 901  GACCGGCCTC CGTCCAGAGA GCTGCTGAAA CATCCGGTCT TCCGTACCAC
 951  GTGGTAG
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Mutations:

pMEVHA-MEKK1-WT (P1045a): Wild type C-terminal kinase domain.
pMEV-MEKK1-K452M (P1045b): Kinase domain as in P1045a but with a mutation (K452M, **AAA** to **ATG**) that results in a kinase-deficient protein.

Selected References:

1. Cuevas,B.D. et al., (2003) EMBO J. 22 (13), 3346-3355
2. Gibson,E.M., et al., (2002) J. Biol. Chem. 277 (12), 10573-10580
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4. Kim,J.W., et al., (2003) J. Biol. Chem. 278 (16), 13995-14001
5. Lange-Carter,C.A., et al., (1993) Science 260 (5106), 315-319
6. Minamino,T., et al., (2002) PNAS USA. 99 (6), 3866-3871
7. Roy,S.K., et al., (2002) PNAS USA. 99 (12), 7945-7950
8. Sadoshima,J., et al., (2002) J. Clin. Invest. 110 (2), 271-279
9. Schlesinger,T.K., et al., (2002) J. Biol. Chem. 277 (12), 10283-10291
10. Yujiri,T., et al., (2003) J. Biol. Chem. 278 (6), 3846-3851
11. Xu, L. et al., (1997) Strategies, 10, 1-3.