

# 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°C if used immediately and store at -20°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 (aminoglycoside 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 25-50 µg/ml Kanamycin (e.g. Cat# LK-1100, Pre-poured 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'...acgct 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.)

MSASQDALP IVPQLQVENGEDI I I IQQDTPETLPGHAKQPYREDAEWL  
 KGQQIGLGFSSCYQAQDVGTLMAV K QVTVYVRNTSSSEQEEVVEALREE  
 IRMMGHLNHPNI I RMLGATCEKSNYNLF I EWMAGGSVAHLLSKYGFAPKES  
 VVINYTEQLLRGLSYLHENQ I I HRDVKGANLLIDSTGQRLR I ADFGAAAR  
 LASKGTGAGFQGLLGT I AFMAPEVLRGQQYGRSCDVSVGCAI I EMAC  
 AKPPWNAEKHSNHLAL I FKIASATTAP S I PSHLSPGLRDVAVRCLLELPQ  
 DRPPSRELLKHVPFR T W

## 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 CTGAGGATGC CCTCCCATC GTCCTCAGC TGCAGGTGGA
51 AAATGGAGAA GATATTATCA TCATTCAGCA GGACACACCA GAAACTCTTC
101 CAGGACATAC CAAAGCGAAA CAGCCTTACA GAGAAGACGC TGAGTGGCTG
151 AAAGGCCAGC AGATAGGCCCT CGGAGCATTT TCTTCCTGTT ACCAAGCACA
201 GGATGTGGGG ACTGGGACTT TAATGGCTGT GAAACAGGTG ACGTACGTCA
251 GAAACACATC CTCCGAGCAG GAGGAGGTGG TGAAGCGTT GAGGGAAGAG
301 ATCCGGATGA TGGGTACCT CAACCATCCA AACATCATCC GGATGCTGGG
351 GGCCACGTGC GAGAAGAGCA ACTACAACCT CTTTCATTGAG TGGATGGCGG
401 GAGGATCTGT GGCTCACCTC TTGAGTAAAT ACGGAGCTTT CAAGGAGTCA
451 GTCGTCATTA ACTACACTGA GCAGTTACTG CGTGGCCTTT CCTATCTCCA
501 CGAGAACCAG ATCATTACCA GAGACGTCAA AGGTGCCAAC CTGCTCATTG
551 ACAGCACCGG TCAGAGGCTG AGAATTGCAG ACTTTGGAGC TGCTGCCAGG
601 TTGGCATCAA AAGGAACCGG TGCAGGAGAG TTCCAGGCAG AGTTACTGGG
651 GACAATTGCA TTCATGGCGC CTGAGGTCTC AAGAGGTGAC CAGTATGGTA
701 GGAGCTGTGA TGTATGGAGT GTTGGCTGCT CCATTATAGA AATGGCTTGT
751 GCAAAACACAC CTTGGAATGC AGAAAAACAC TCCAATATC TCGCCTTGAT
801 ATTTAAGATT GCTAGCGCAA CTACTGCACC GTCCATCCC TCACACCTGT
851 CCCCGGTCT GCGGACGTG GCCGTGCGCT GCTTAGAACT TCAGCCTCAG
901 GACCGGCTC 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
3. Ito, M., et al., (1999) Mol. Cell. Biol. 19 (11), 7539-7548 (1999)
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.