

MEK1 ProtéGene™ Set

Catalog# P1030
 Lot# 171021

Materials Provided:

1. pMEV-MEK1-WT (P1030a): 20µg in 40µl TE (pH7.5), 0.5mg/ml.
2. pMEV-MEK1-DN (P1030b): 20µg in 40µl TE (pH7.5), 0.5mg/ml.
3. pMEV-MEK1-CA (P1030c): 20µg in 40µl TE (pH7.5), 0.5mg/ml.
4. Product Information Sheets.

Note: Individual plasmids can be ordered separately. Also available is the kinase-deficient mutant pMEV-MEK1-K97R (Cat#P1030d). 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°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 transformed with the plasmids should be maintained and grown in media containing 25-50µg/ml Kanamycin (e.g. #LK-1100, Prepared 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 MEK1 and Mutants

MEK1 and MEK2 are members of the dual specificity protein kinase family, which acts as a mitogen-activated protein (MAP) kinase kinase. MAP kinases, also known as extracellular signal-regulated kinases (ERKs), act as an integration point for multiple biochemical signals. MEK1 lies upstream of MAP kinases and stimulates the enzymatic activity of MAP kinases upon wide variety of extra- and intracellular signals. MEKs are involved in many cellular processes such as proliferation, differentiation, transcription regulation and development.

Several residues are essential for MEK to function: K97 for its catalytic activity, S218 and S222 for activation by upstream activators. The dominant negative mutant provided (P1030b) contains 3 point mutations (K97R; S218A; S222A) and can neither be phosphorylated by its activators nor phosphorylate its downstream effectors (ERKs). The constitutively active mutant (P1030c) contains 2 point mutations (S218E and S222E) and a deletion of amino acid residues 31-52.^(1,6,9) The resulting protein is ~400 times more active than recombinant MEK1 wild type expressed in *E. coli*, and is active without the need of being phosphorylated by its upstream activators.

Molecular Features of the Inserts:

Gene: *Homo sapiens* mitogen-activated protein kinase kinase 1
 GenBank Reference Sequence: NM_002755
 5'-Cloning Site: Bam HI
 5'-Junction Sequence: 5'...tac gct gga tcc ATG CCC AAG...3'
 3'-Cloning Site: Bam HI
 3'-Junction Sequence:
 5'...gcgggatcctcttggcttccaaccac TTA GAC GCC...-3'

hMEK1 Protein Sequence

(393 amino acid residues. Amino acid residues 31-52, K97, S218, S222 are in bold and underlined.)

MPKKKPTPIQLNPAPDGSVAVNGTSSAETNLEALQKLELELDEQQRKR
 LEAFLTQKQKVGELKDDDFEKISELGAGNGGVVFKVSHKPSGLVMARKLIHLEI
 KPDIRNQI IRELQVLHECNSPYIVGFYGFYSDGEIISICMEHMDGGSLDQVLKK
 AGRIPEQILGKVSIAVAVIKGLTYLREKHKIMHRDVKPSPNIIVNSRGEIKLCDFGV
 SGQLIDSMANSFVGTSTRYSMPERLQTHYSVQSDIWSMGLSLVEMAVGRYP IPP
 PDAKELELMFQGVQVEGDAETPPRPRTPGRPLSSYGMDSRPPMAIFELLDYIVN
 EPPPKLPSPGVFSLFQDFVNKCLIKNPAERADLKQLMVHAFIKRSDAEEVDFAG
 WLCSTIGLNQPSPTTHAAGV

hMEK1 Nucleotide Sequence

(1182bps. Nucleotides encoding aa32-51, K97, S218, S222 are in bold and underlined)

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1  ATGCCCAAGA AGAAGCCGAC GCCCATCCAG CTGAACCCGG CCCCCGACGG
51  CTCTGCAGTT AACGGGACCA GCTCTGCGGA GACCAACTTG GAGGCCTTGC
101 GAGGCGCTTTC TTACCCAGAA GCAGAAGGTT GGAGAAGTGA AGGATGACGA
151 GAGGCGCTTTC TTACCCAGAA GCAGAAGGTT GGAGAAGTGA AGGATGACGA
201  CTTTGAGAAAG ATCAGTGAGC TGGGGGCTGG CAATGGCCGT GTGGTGTTC
251  AGGTCTCCCA CAAGCCTTCT GCCTGGTCA TGGCCAGAAAA GCTAATTCAT
301  CTGGAGATCA AACCCGCAAT CCGGAACCAG ATCATAAGGG AGTGCAGGT
351  TCTGCATGAG TGCAACTTCT CGTACATCGT GGGCTTCTAT GGTGCGTCT
401  ACAGCGATGG CGAGATCAGT ATCTGCATGG AGCACATGGA TGGAGGTTCT
451  CTGGATCAAG TCCTGAAGAA AGCTGGAAGA ATTCCTGAAC AAATTTTAGG
501  AAAAGTTAGC ATTGCTGTAA TAAAAGGCCCT GACATATCTG AGGGAGAAGC
551  ACAAGATCAT GCACAGAGAT GTCAAGCCCT CCAACATCCT AGTCAACTCC
601  CGTGGGGAGA TCAAGCTCTG TGACTTTGGG GTCAGCGGGC AGCTCATCGA
651  CTCCATGGCC AACTCCTTCG TGGGCACAAG GTCCTACATG TCCGACGAAA
701  GACTCCAGGG GACTCATTAC TCTGTGCGT CAGACATCTG GAGCATGGGA
751  CTGTCTCTGG TAGAGATGGC GGTFTGGGAG TATCCCATCC CTCCTCCAGA
801  TGCCAAGGAG CTGGAGCTGA TGTTTGGGTG CCAGTGTGAA GGAGATCCGG
851  CTGAGACCCC ACCCAGGCCA AGGACCCCCT GGAGGCCCTC TAGTTCATAC
901  GGAATGGACA GCCGACCTCC CATGGCAATT TTTGAGTTGT TGGATTACAT
951  AGTCAACGAG CCTCCTCCAA AACTGCCCAG TGGAGTGTTT AGTCTGGAA
1001 TCAAGATTT TGTGAATAAA TGCTTAATAA AAAACCCCGC AGAGAGAGCA
1051 GATTGGAAGC AACTCATGGT TCATGCTTTT ATCAAGAGAT CTGATGCTGA
1101 GGAAGTGGAT TTTGCAGGTT GGCTCTGCTC CACCATCGGC CTTAACCAGC
1151 CCAGCACACC AACCCATGCT GCTGGCGTCT AA
  
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Mutations:

pMEV-MEK1-WT (P1030a): No mutation
 pMEV-MEK1-DN (P1030b): K97R; S218A; S222A
 pMEV-MEK1-CA (P1030c): Δ32-51; S218E; S222E
 pMEV-MEK1-K97R(P1030d): K97R

References:

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7. Robbins D. J., et al., 1993, *J. Biol Chem.*, 268: 5097-5106
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