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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^{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 (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, 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 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'-…gcgggatcctctttgttgcttcccaaccac **TTA GAC GCC**…-3'

hMEK1 Protein Sequence

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

MPKKKPTPIQLNPAPDGSAVNGTSSAETNLEALQKKLEELELDEQQRKR
LEAFLTQKQKVGELKDDDFEKISELGAGNGGVVFKVSHKPSGLVMARKLIHLEI
KPAIRNQIIRELQVLHECNSPYIVGFYGAFYSDGEISICMEHMDGGSLDQVLKK
AGRIPEQILGKVSIAVIKGLTYLREKHKIMHRDVKPSNILVNSRGEIKLCDFGV
SGQLIDSMANSFVGTRSYMSPERLQGTHYSVQSDIWSMGLSLVEMAVGRYPIPP
PDAKELELMFGCQVEGDAAETPPRPRTPGRPLSSYGMDSRPPMAIFELLDYIVN
EPPPKLPSGVFSLEFQDFVNKCLIKNPAERADLKQLMVHAFIKRSDAEEVDFAG
WLCSTIGLNQPSTPTHAAGV

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  AGAAGAAGCT GGAGGAGCTA GAGCTTGATG AGCAGCAGCG AAAGCGCCTT
 151  GAGGCCTTTC TTACCCAGAA GCAGAAGGTG GGAGAACTGA AGGATGACGA
 201  CTTTGAGAAG ATCAGTGAGC TGGGGGCTGG CAATGGCGGT GTGGTGTTCA
 251  AGGTCTCCCA CAAGCCTTCT GGCCTGGTCA TGGCCAGAAA GCTAATTCAT
 301  CTGGAGATCA AACCCGCAAT CCGGAACCAG ATCATAAGGG AGCTGCAGGT
 351  TCTGCATGAG TGCAACTCTC CGTACATCGT GGGCTTCTAT GGTGCGTTCT
 401  ACAGCGATGG CGAGATCAGT ATCTGCATGG AGCACATGGA TGGAGGTTCT
 451  CTGGATCAAG TCCTGAAGAA AGCTGGAAGA ATTCCTGAAC AAATTTTAGG
 501  AAAAGTTAGC ATTGCTGTAA TAAAAGGCCT GACATATCTG AGGGAGAAGC
 551  ACAAGATCAT GCACAGAGAT GTCAAGCCCT CCAACATCCT AGTCAACTCC
 601  CGTGGGGAGA TCAAGCTCTG TGACTTTGGG GTCAGCGGGC AGCTCATCGA
 651  CTCCATGGCC AACTCCTTCG TGGGCACAAG GTCCTACATG TCGCCAGAAA
 701  GACTCCAGGG GACTCATTAC TCTGTGCAGT CAGACATCTG GAGCATGGGA
 751  CTGTCTCTGG TAGAGATGGC GGTTGGGAGG TATCCCATCC CTCCTCCAGA
 801  TGCCAAGGAG CTGGAGCTGA TGTTTGGGTG CCAGGTGGAA GGAGATGCGG
 851  CTGAGACCCC ACCCAGGCCA AGGACCCCCG GGAGGCCCCT TAGCTCATAC
 901  GGAATGGACA GCCGACCTCC CATGGCAATT TTTGAGTTGT TGGATTACAT
 951  AGTCAACGAG CCTCCTCCAA AACTGCCCAG TGGAGTGTTC AGTCTGGAAT
1001  TTCAAGATTT TGTGAATAAA TGCTTAATAA AAAACCCCGC AGAGAGAGCA
1051  GATTTGAAGC 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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8. Zheng C. F. and Guan K. L., 1993, *J. Biol Chem,* 268: 11435-11439
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