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# BRAF ProtéGene™ Set

**Catalog#** P1060
**Lot#** On vial label

**Materials Provided**

1. pMEV-BRAF-WT (P1060a): 20 μg
2. pMEV-BRAF-K482M (P1060b): 20 μg
3. pMEV-BRAF-ED (P1060c): 20 μg
4. pMEV-BRAF-V599E (P1060d): 20 μg
5. Product Information Sheets.

***Note:*** *Individual plasmids can be ordered separately. Some plasmids are shipped as lyophilized pellet.*

**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.

**Expression Vector:**

pMEV-2HA: Cat# P1001.

**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 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 BRAF and Mutants**

Genes of the RAF family encode three cytoplasmic serine/threonine protein kinases that are regulated by RAS and mediate cellular responses to growth signals from the cell surface to the nuclear. Human BRAF was first cloned from a testis cDNA library.[3] Mutations of BRAF gene have been found in 66% of melanomas and many other types of cancers. All mutations reside in the kinase domain, with a single substitution, V599E accounting for 80%.[1, 2] K482 is a highly conserved active-site residue. BRAF, and all other kinases, mutated at this position are inactive as a protein kinase. T598 and S601 reside within kinase subdomains VII and VIII and are activating phosphorylation sites. Replacement of these two sites by acidic residues (BRAF-ED) renders BRAF constitutively active.[4]

**Molecular Features of the Inserts:**

Gene: *H. sapiens* v-raf sarcoma viral oncogene homolog B1
GenBank Reference Sequence: NM_004333
5'-Cloning Site: Xba I; 3'-Cloning Site: Sal I
5'-Junction Sequence (upper strand):
5'-tac gct tgg atc cgc ggc aag cct cga gaa ttc acg cgt ggt acc **tct aga ATG GCG GCG**-3'
3'-Junction Sequence (lower strand):
5'-…cccgg**gtcgacgaattc GGGAAAA**…-3'

**BRAF Insert Nucleotide Sequence**

*(Insert size: 2467 bps, including 3'-UTR sequences. K482, T598, S601, V599 are in bold and underlined.)*

```
   1  atggcggcgc tgagcggtgg cggtggtggc ggcgcggagc cgggccaggc tctgttcaac
       m  a  a   l  s  g   g  g  g  g   g  a  e   p  g  q   a  l  f  n
  61  ggggacatgg agcccgaggc cggcgccggc gccggcgccg cggcctcttc ggctgcggac
       g  d  m   e  p  e   a  g  a  g   a  g  a   a  a  s   s  a  a  d
 121  cctgccattc cggaggaggt gtggaatatc aaacaaatga ttaagttgac acaggaacat
       p  a  i   p  e  e   v  w  n  i   k  q  m   i  k  l   t  q  e  h
 181  atagaggccc tattggacaa atttggtggg gagcataatc caccatcaat atatctggag
       i  e  a   l  l  d   k  f  g  g   e  h  n   p  p  s   i  y  l  e
 241  gcctatgaag aatacaccag caagctagat gcactccaac aaagagaaca acagttattg
       a  y  e   e  y  t   s  k  l  d   a  l  q   q  r  e   q  q  l  l
 301  gaatctctgg ggaacggaac tgatttttct gtttctagct ctgcatcaat ggataccgtt
       e  s  l   g  n  g   t  d  f  s   v  s  s   s  a  s   m  d  t  v
 361  acatcttctt cctcttctag cctttcagtg ctaccttcat ctctttcagt ttttcaaaat
       t  s  s   s  s  s   s  l  s  v   l  p  s   s  l  s   v  f  q  n
 421  cccacagatg tggcacggag caaccccaag tcaccacaaa aacctatcgt cagagtcttc
       p  t  d   v  a  r   s  n  p  k   s  p  q   k  p  i   v  r  v  f
 481  ctgcccaaca aacagaggac agtggtacct gcaaggtgtg gagttacagt ccgagacagt
       l  p  n   k  q  r   t  v  v  p   a  r  c   g  v  t   v  r  d  s
 541  ctaaagaaag cactgatgat gagaggtcta atcccagagt gctgtgctgt ttacagaatt
       l  k  k   a  l  m   m  r  g  l   i  p  e   c  c  a   v  y  r  i
 601  caggatggag agaagaaacc aattggttgg gacactgata tttcctggct tactggagaa
       q  d  g   e  k  k   p  i  g  w   d  t  d   i  s  w   l  t  g  e
 661  gaattgcatg tggaagtgtt ggagaatgtt ccacttacaa cacacaattt tgtacgaaaa
       e  l  h   v  e  v   l  e  n  v   p  l  t   t  h  n   f  v  r  k
 721  acgtttttca ccttagcatt ttgtgacttt tgtcgaaagc tgcttttcca gggtttccgc
       t  f  f   t  l  a   f  c  d  f   c  r  k   l  l  f   q  g  f  r
 781  tgtcaaacat gtggttataa atttcaccag cgttgtagta cagaagttcc actgatgtgt
       c  q  t   c  g  y   k  f  h  q   r  c  s   t  e  v   p  l  m  c
 841  gttaattatg accaacttga tttgctgttt gtctccaagt tctttgaaca ccacccaata
       v  n  y   d  q  l   d  l  l  f   v  s  k   f  f  e   h  h  p  i
 901  ccacaggaag aggcgtcctt agcagagact gccctaacat ctggatcatc cccttccgca
       p  q  e   e  a  s   l  a  e  t   a  l  t   s  g  s   s  p  s  a
 961  cccgcctcgg actctattgg gccccaaatt ctcaccagtc cgtctccttc aaaatccatt
       p  a  s   d  s  i   g  p  q  i   l  t  s   p  s  p   s  k  s  i
1021  ccaattccac agcccttccg accagcagat gaagatcatc gaaatcaatt tgggcaacga
       p  i  p   q  p  f   r  p  a  d   e  d  h   r  n  q   f  g  q  r
1081  gaccgatcct catcagctcc caatgtgcat ataaacacaa tagaacctgt caatattgat
       d  r  s   s  s  a   p  n  v  h   i  n  t   i  e  p   v  n  i  d
1141  gacttgatta gagaccaagg atttcgtggt gatggaggat caaccacagg tttgtctgct
       d  l  i   r  d  q   g  f  r  g   d  g  g   s  t  t   g  l  s  a
1201  acccccccctg cctcattacc tggctcacta actaacgtga aagccttaca gaaatctcca
       t  p  p   a  s  l   p  g  s  l   t  n  v   k  a  l   q  k  s  p
1261  ggacctcagc gagaaaggaa gtcatcttca tcctcagagg acaggaatcg aatgaaaaca
       g  p  q   r  e  r   k  s  s  s   s  s  e   d  r  n   r  m  k  t
1321  cttggtagac gggactcgag tgatgattgg gagattcctg atgggcagat tacagtggga
       l  g  r   r  d  s   s  d  d  w   e  i  p   d  g  q   i  t  v  g
1381  caaagaattg gatctggatc atttggaaca gtctacaagg gaaagtggca tggtgatgtg
       q  r  i   g  s  g   s  f  g  t   v  y  k   g  k  w   h  g  d  v
1441  gcagtgAAAa tgttgaatgt gacagcacct acacctcagc agttacaagc cttcaaaaat
       a  v  k   m  l  n   v  t  a  p   t  p  q   q  l  q   a  f  k  n
1501  gaagtaggag tactcaggaa aacacgacat gtgaatatcc tactcttcat gggctattcc
       e  v  g   v  l  r   k  t  r  h   v  n  i   l  l  f   m  g  y  s
1561  acaaagccac aactggctat tgttacccag tggtgtgagg gctccagctt gtatcaccat
       t  k  p   q  l  a   i  v  t  q   w  c  e   g  s  s   l  y  h  h
1621  ctccatatca ttgagaccaa atttgagatg atcaaactta tagatattgc acgacagact
       l  h  i   i  e  t   k  f  e  m   i  k  l   i  d  i   a  r  q  t
1681  gcacagggca tggattactt acacgccaag tcaatcatcc acagagacct caagagtaat
       a  q  g   m  d  y   l  h  a  k   s  i  i   h  r  d   l  k  s  n
1741  aatatatttc ttcatgaaga cctcacagta aaaataggtg attttggtct agctACAGTG
       n  i  f   l  h  e   d  l  t  v   k  i  g   d  f  g   l  a  T  V
1801  aaaTCTcgat ggagtgggtc ccatcagttt gaacagttgt ctggatccat tttgtggatg
       k  S  r   w  s  g   s  h  q  f   e  q  l   s  g  s   i  l  w  m
1861  gcaccagaag tcatcagaat gcaagataaa aatccataca gctttcagtc agatgtatat
       a  p  e   v  i  r   m  q  d  k   n  p  y   s  f  q   s  d  v  y
1921  gcatttggga ttgttctgta tgaattgatg actggacagt tacctattc aaacatcaac
       a  f  g   i  v  l   y  e  l  m   t  g  q   l  p  y   s  n  i  n
1981  aacagggacc agataatttt tatggtggga cgaggatacc tgtctccaga tctcagtaag
       n  r  d   q  i  i   f  m  v  g   r  g  y   l  s  p   d  l  s  k
2041  gtacggagta actgtccaaa agccatgaag agattaatgg cagagtgcct caaaaagaaa
       v  r  s   n  c  p   k  a  m  k   r  l  m   a  e  c   l  k  k  k
2101  agagatgaga gaccactctt tccccaaatt ctcgcctcta ttgagctgct ggcccgctca
       r  d  e   r  p  l   f  p  q  i   l  a  s   i  e  l   l  a  r  s
2161  ttgccaaaaa ttcaccgcag tgcatcagaa ccctccttga atcgggctgg tttccaaaca
       l  p  k   i  h  r   s  a  s  e   p  s  l   n  r  a   g  f  q  t
2221  gaggatttta gtctatatgc ttgtgcttct ccaaaaacac ccatccaggc agggggatat
       e  d  f   s  l  y   a  c  a  s   p  k  t   p  i  q   a  g  g  y
2281  ggtgcgtttc ctgtccactg aaacaaatga gtgagagagt tcaggagagt agcaacaaaa
       g  a  f   p  v  h   -
2341  ggaaaataaa tgaacatatg tttgcttata tgttaaattg aataaaatac tctctttttt
2401  tttaaggtga aaaaaaaaaa aaaaaaaaaa aaaaaaaaaa aaaaaaaaaa aacccgaatt
2461  cgtcgac
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**Mutations:**

pMEV-BRAF-WT (P1060a): No mutation
pMEV-BRAF-K482M (P1060b): K482M, AAA→ATG
pMEV-BRAF-ED (P1060c): T598E(ACA→GAA), S601D(TCT→GAT)
pMEV-BRAF-V599E (P1060d): V599E (GTG→GAG)

**Selected References:**

1. Davies, H. et al., 2002. Mutations of the BRAF gene in human cancer. *Nature* 417: 949-954,
2. http://www.ncbi.nlm.nih.gov/entrez/dispomim.cgi?id=164757
3. Sithanandam, G. et al., 1990. Complete coding sequence of a human B-raf cDNA and detection of B-raf protein kinase with isozyme specific antibodies. *Oncogene* 5: 1775-1780, 1990.
4. Zhang,B.H. and Guan,K.L. (2000) Activation of B-Raf kinase requires phosphorylation of the conserved residues thr598 and ser601. *EMBO J.*, **19**, 5429–5439.