

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°C if used immediately and store at -20°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 (aminoglycoside 3' phosphotransferase) 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 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 aqa ATG GCG GCG-3'

3'-Junction Sequence (lower strand):

5'-...cccgggtcgaqaattc GGGAAAA...-3'

BRAF Insert Nucleotide Sequence

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

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1 atggcggcgc tgagcgggtg cggtgggtgg ggcgcgggagc cgggccaaggc tctgttcaac
   m a a l s g g g g g g g a e p g g q a l f n
61 ggggacatgg agccccaggc cggcgcgggc gccgcggcgc cggcctcttc ggctgggac
   g d m e p e a g a g a g a a a s s a a d
121 cctgcatttc cggaggaggt gtggaatc aaacaatga ttaagttac acaggaacat
   p a i p s e e v w n i k g m i k l t c e h
181 atagagcccc tahtggacaa atttggtagg gagcataac caccatcaat atactgtggag
   i e a l l d k f g g e h n p p s i y l e
241 gctatgaag aatacaaccg caagctagat gcactccaac aaagagaaca acagtattg
   a y e e y t s k l d a l l g q r e g q l l
301 gaattctcgg ggaacggaac tgattttctt gtttctagct ctgcaatcaat ggataccgct
   e s l g n g t d f s v s s s a s m d t v
361 acattctctt cctctctcag ccttctcagtc ctactctcat cctctcagtc ttttcaaat
   t s s s s s s l s v l p s s l s v f g n
421 cccacagatg tggcaccggg caaccccaag tcaccacaac aacctatcgt cagagtcttc
   p t d v a r s n p k s p q k p i v r v f
481 ctgccaaca aacagaggac agtggtaact gcaaggtgtg gagttacagt ccgagaacagt
   l p n k q r t v v p a r c g v t v r d s
541 ctaaaagaa gactgatgat gagaggtcta atcccagagt cgtgtgctgt ttaacagaatt
   l k k a l m m r g l i p e c c a v y r i
601 cagatggag agaagaaac aattggttg gcaactgata tttcttgct tactggagaa
   q d g e k k p i g w d t d i s w l t g e
661 gaattgcatg tggagatggt ggagaatgt ccaactcaaa cacacaattt tgtagcaaaa
   e l h v e v l e n v p l t t h n f v r k
721 acgttttca ccttagcatt ttgtgacttt tgtagaagg tgcttttcca gggttccgc
   t f f t l a f c d f c r k l l f q g f r
781 ttcaaacat gtggttataa atttccaag cgtttgagta cagaagttcc actgattgtt
   c q t c g y k f h q r c s t e v p l m c
841 gtttaattg accaacttga ttgtgcttt gtctccaagt tctttgaaca ccaccaata
   v n y d g l d l l f v s k f f e h h p i
901 ccaaggaag aggcctcctt agcagagact gccctaacat ctggatcact ccctccgca
   p g e e a s l a e t a l t s g s s p s a
961 ccgcctcgg actctattgg gccccaatt ctcaaccgct cgtctcttc 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 gaagatcact 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 caattatgat
   d r s s s a p n v h i n t i e p v n i d
1141 gaattgatta gagaccaagg atttctggt gatggaggat caaccacagg ttgtgtcct
   d l i r d q g f r g d g g s t t g l s a
1201 acccccctg cctcattacc tggctcacta actaactaga aagcttaca gaactctcca
   t p p a s l p g s l t n v k a l g k s p
1261 ggactccag gagaagaaga gtcatcttca tctctcagag acaggaatcg aatgaaaca
   g p q r e r k s s s s g s e d r n r m k t
1321 cttggtagac gggactcga gtagatggt gagattcctg atgggcaagt tacagtggga
   l g r r d s s d d w e i p d d g q i t v g
1381 caaagaattg gatctggatc atttggaaac gtctcaagg gaaagtggca tgggtgatgt
   q r i g s g s f g t v y g k g k w h g d v
1441 gcagtgaag ttttgaatgt gacagcactc acactcagc agttacaagc cttcaaaatt
   a v k m l n v t a p t p q q l g a f k n
1501 gaagtggag tactcaggaa aacacgacat ctgtaattcc tactcttcat gggctattcc
   e v g v l r k t r h v n i l l f m g y s
1561 acaagccac aactgcttat ttttccagag tgggtggagg gctccagctt gtaccacat
   t k p q l a i v t q w c e g s s l y h h
1621 ctccatata ttgagaccaa atttggatgt atcaaaccta tagatattgc acgacagact
   l h i i e t k f e m i k l i d i a r q t
1681 gcaagggca tggattactt caacgccaag tcaatcttc acagagaact caagagtaat
   a g g m d y l h a k s i l h r d l k s n
1741 aatatattc ttcattgaga cctcacagta aaaaatggtg attttgctct agctACGCG
   n i f l h e d l t v k i g d f g l a T V
1801 aaACTCGat ggagtggttc ccatcagitt gaacactgtt ctggatcact ttgtggatg
   k S r w s g s h q f e e q l s g s i l w m
1861 gcaaccaga gtcactcaga gcaagataaa aatccataca gcttctcagc agatgtatat
   a p e v i r m g d k n p y s f g s d v y
1921 gcatttggga ttgtctctga tgaattgatg actggcagat tactcttacc aaacatcaac
   a f g i v l y e l m t g g l p y s n i n
1981 aacagggaac agataatttt tatggtggga cgaggatacc tgtctcaga tctcagtaag
   n r d q i i f m v g r g y l s p d l s k
2041 gtaacggagta actgtccaaa agccatgaag agatataatg cagagtgcct caaaaagaaa
   v r s n c p k a m k r l m a e c l k k g
2101 agagatgaga gaccactctt tccccaaatt ctgcctctca ttgagctgct ggcccctca
   r d e r p l f p q i l a s i e l l a r s
2161 ttgccaaaaa ttaccgacag tgcatacaga cctccttga atcggctgct ttccaaca
   l p k i h r s a s e p s l n r a g f q t
2221 gaggatttta gcttatatgc ttgtgctctt ccaaaaaaac ccatccaggc aggggatgat
   e d f s l y a c a s p k t p i g a g g y
2281 ggtggcttc ctgtccactg aaacaatga gtgagagagt tcaggagagt agcaacaaa
   g a f p v h
2341 ggaataaaa tgaacatag ttgtctata tgttaattg aataaaatac tctcttttt
2401 ttaaggtag aaaaaaaaa aaaaaaaaa aaaaaaaaa aaaaaaaaa aaccgcaatt
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/dispmom.cgi?d=164757>
3. Sihanandam, 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.