

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 cggcgcggcgc gccgcggcgc cggcctcttc ggctgggac
g d m e p e a g a g a g a a a s s a a d
121 cctgcattc 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 atagagccc taatggacaa atttggtagg gagcataac caccatcaat atactgttag
i e a l l d k f g g e h n p p s i y l e
241 gctatgaag aatacaaccg caagctagat gcactcaaac 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 tgattttct gtttctagct ctgcaatcaat ggataccgtt
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 ctcttctcagt 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 cacagtcttc
p t d v a r s n p k s p q k p i v r v f
481 ctgccacaac 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 ctaaagaaag cactgatgat 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 caggatggag agaagaaac aattggttg ggaactgata tttcttgct tactggagaa
g d g e k k p i g w d t d i s w l t g e
661 gaattgcatg tggagatggt ggagaatgt ccaactcaac 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 tgtagaagc tgcttttcca gggttccgc
t f f t l a f c d f c r k l l f q g f r
781 ttcaaacatc gtggttataa atttccacag cgtttgtagt cagaagttcc actgattgtt
c q t c g y k f h q r c s t e v p l m c
841 gtttaattatg 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 ccaacggaag 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 ccgcctcggg actctattgg gccccaatt ctcaacagtc cgtctcttc aaaatcaatt
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 atttctggtt gtagggagat 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 ttgctcacta actaactaga aagcttaca gaactctcca
t p p a s l p g s l t n v k a l g k s p
1261 ggacctcagc gagaagaaga gtcatcttca tctctcagag acaggaatcg aatgaaaca
g p q r e r k s s s s g e d r n r m k t
1321 cttggtagac gggactcgaag tgatgattgg gagattcctg atgggcagat 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 gtctcaacgg gaaagtggca tgggttagtg
q r i g s g s f g t v y k s g k w h g d v
1441 gcagtgaaga tgttgaatgt gacagcactc acactcagc agttacaagc cttcaaaatt
a v k m l n v t a p t p q g l q a f k n
1501 gaagtggagg 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 acaaaagcag aactgcttat ttttaccagg tgggtggagg gctccagctt gtaccacatc
t k p q l a i v t g w c e g s s l y h h
1621 ctccatatac ttgagaccaa atttgagagt atcaaaccta tagatattgc acgacagact
l h i i e t k f e m i k l i d i a r q t
1681 gcaacgggca tggattactt caacgccaag tcaatctcc 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 agct ACGCG
n i f l h e d l t v k i g d f g l a t v
1801 aaatctcgat ggaagtggtc ccatcagitt gaacagttgt ctggatcact ttgtggatg
k s r w s g s h q f e q l s g s i l w m
1861 gcaacgaagc tcatcagaat gcaagataaa aatccataca gcttctcagtc 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 actggcagct tactcttacc aaacatcaac
a f g i v l y e l m t g g l p y s n i n
1981 aacagggacc 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 cacagtgcct 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 ttccaccgag tgcatacaga cctccttga atcggctgtg 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 agggggatag
e d f s l y a c a s p k t p i g a g g y
2281 ggtggcttc ctgtccactg aaacaatgag gtgagagagt tcaggagagt agcaacaaa
g a f p v h
2341 ggaataataa tgaacatag ttgtctata tgttaaatg aataaaatac tctctttttt
2401 ttaaggtga aaaaaaaaa aaaaaaaaa aaaaaaaaa aaaaaaaaa aaccogaatt
2461 cgtcagc
    
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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.