

ProtéGene™ Gene Sets K-Ras 2B

Cat# P1025
 Lot# 303091

Materials Provided:

1. KRAS2B-WT (P1025a): 20 µg plasmid DNA in 40 µl TE.
2. KRAS2B-S17N (P1025b): 20 µg plasmid DNA in 40 µl TE.
3. KRAS2B-G12V (P1025c): 20 µg plasmid DNA in 40 µl TE.
4. Product Information Sheets.

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

Receiving and Storage:

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. 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' 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 25-50 µg/ml Kanamycin (e.g. #LK-1100, Prepoured 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 KRAS2B and Mutants

Ras proteins are members of small GTPase superfamily. They bind GDP/GTP and possess intrinsic GTPase activity. These proteins act as switches to turn on/off their downstream target proteins in response to upstream stimuli because they can alternate between an inactive form bound to GDP and an active form bound to GTP. Activating mutations of KRAS2, including G12V, have been implicated in various malignancies, including lung denocarcinoma, mucinous adenoma, ductal carcinoma of the pancreas and colorectal carcinoma. On the other hand, S17N is a widely used dominant negative mutant.

Alternative splicing of KRAS-2 gene leads to variants encoding two isoforms that differ in the C-terminal region. KRAS-2B variant terminates in exon 4b, encoding a unique C terminus and lacks exon 4a that is present in the longer transcript variant KRAS-2A.

Molecular Features of the Inserts:

Gene: *Homo sapiens* v-Ki-ras2 Kirsten rat sarcoma 2 viral oncogene homolog (KRAS2), transcript variant B

Other names: Transforming protein p21b, K-Ras 2B, Ki-Ras, c-K-ras .

GenBank Reference Sequence: NM_004985

Protein Accession: P01118

5'-Cloning Site: Bam HI

5'-Junction Sequence: 5'-...tac gct gga tcc ATG ACT GAA-...3'

3'-Cloning Site: Xho I

3'-Junction Sequence (lower strand):
 5'-...tga att ctc gag TTA CAT AAT...-3'

KRAS2B Protein Sequence

(188 amino acid residues. Amino acid residues G12 and S17 are in bold and underlined.)

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1 MTEYKLVVVV AGGVGKSALT IQLIQNHFVD EYDPTIEDSY
41 RKQVVLDGET CLLDILDTAG QEYVSAMRDQ YMRTGEGFLC
81 VFAINNTKSF EDIHHYREQI KRVKDSEDVP MVLVGNKCDL
121 PSRTVDTKQA QDLARSYGIP FIETSAKTRQ GVDDAFYTLV
161 REIRKHKEKM SKDGKKKKKKK SKTKCVIM
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KRAS2B Nucleotide Sequence

(567bps. Nucleotides encoding G12 and S17 are in bold and underlined)

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1 ATGACTGAAT ATAACTTGT GGTGGTTGGA GCTGGTGGCG TAGGCAAGAG
51 TGCCTTGACG ATACAGCTAA TTCAGAATCA TTTTGTGGAC GAATATGATC
101 CAACAATAGA GGATTCCTAC AGGAAGCAAG TAGTAATTGA TGGAGAAACC
151 TGTCCTTGG ATATTCTCGA CACAGCAGGT CAAGAGGAGT ACAGTGCAAT
201 GAGGGACCAG TACATGAGGA CTGGGGAGGG CTTTCTTGT GTATTTGCCA
251 TAAATAATAC TAAATCATT GAAGATATTC ACCATTATAG AGAACAAATT
301 AAAAGAGTTA AGGACTCTGA AGATGTACCT ATGGTCCTAG TAGGAAATAA
351 ATGTGATTTG CCTTCTAGAA CAGTAGACAC AAAACAGGCT CAGGACTTAG
401 CAAGAAGTTA TGAATTCTCT TTTATTGAAA CATCAGCAAA GACAAGACAG
451 GGTGTTGATG ATGCCTTCTA TACATTAGTT CGAGAAATTC GAAAAATAA
501 AGAAAAGATG AGCAAAGATG GTAAAAAGAA GAAAAGAAG TCAAAGACAA
551 AGTGTGTAAT TATGTAA
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Mutations:

KRAS2B-WT (P1025a): No mutation
 KRAS2B-S17N (P1025b): AGT to AAT
 KRAS2B-G12V (P1025c): GGT to GTT

References:

The following GenBank/ Swiss-Prot entries are the reference sequences compiled from various individual entries from many laboratories. They both have a regularly updated collection of references.

GenBank Entry: [NM_004985](http://www.ncbi.nlm.nih.gov/nuccore/NM_004985)

Swiss-Prot Entry: [P01118](http://www.expasy.org/entry/P01118)