

ProtéGene™ Gene Sets NRAS

Cat# P1015
 Lot# On vial label

Materials Provided:

1. NRAS-WT (P1015a): 20 µg plasmid DNA in 40 µl TE.
2. NRAS-S17N (P1015b): 20 µg plasmid DNA in 40 µl TE.
3. NRAS-G12V (P1015c): 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 NRAS 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. Human NRAS cDNA was first cloned from human sarcoma cell lines (HT1080 and RD). Activating mutations of NRAS at codons 12, 13 or 62 were found in a variety of human tumors. S17N is a widely used dominant negative mutant.

Molecular Features of the Inserts:

Gene: *Homo sapiens* neuroblastoma RAS viral (v-ras) oncogene homolog

Other names: N-ras; NRAS1; neuroblastoma RAS viral (v-ras) oncogene homolog; v-ras neuroblastoma RAS viral oncogene homolog; Transforming protein N-Ras.

GenBank Reference Sequence: NM_002524

Protein Accession:

5'-Cloning Site: Bam HI

5'-Junction Sequence: 5'-...tac gct **gga tcc ATG ACT GAG TA**...3'

3'-Cloning Site: Eco RI

3'-Junction Sequence (lower strand):

5'-...**tga att c TTA CAT CAC CAC**...-3'

NRAS Protein Sequence

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

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1 MTEYKLVVVG AGGVGKSALT IQLIQNHFVD EYDPTIEDSY
41 RKQVVIDGET CLLDILDITAG QEEYSAMRDQ YMRTGEGFLC
81 VFAINNSKSF ADINLYREQI KRVKDSDVDP MVLVGNKCDL
121 PTRTVDTKQA HELAKSYGIP FIETSAKTRQ GVEDAFYTLV
161 REIRQYRMKK LNSSDDGTQG CMGLPCVVM
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NRAS Nucleotide Sequence

(570bps. Nucleotides encoding G12 and S17 are in bold and underlined)

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1 ATGACTGAGT ACAAACTGGT GGTGGTTGGA GCAGGTGGTG TTGGGAAAAG
51 CGCACTGACA ATCCAGCTAA TCCAGAACCA CTTTGTAGAT GAATATGATC
101 CCACCATAGA GGATTCTTAC AGAAAACAAG TGGTTATAGA TGGTGAAACC
151 TGTTTGTGG ACATACTGGA TACAGCTGGA CAAGAAGAGT ACAGTGCCAT
201 GAGAGACCAA TACATGAGGA CAGGCGAAGG CTTCTCTGT GTATTTGCCA
251 TCAATAATAG CAAGTCATT GCGGATATTA ACCTCTACAG GGAGCAGATT
301 AAGCGAGTAA AAGACTCGGA TGATGTACCT ATGGTGCTAG TGGGAAACAA
351 GTGTGATTTT CCAACAAGGA CAGTTGATAC AAAACAAGCC CACGAACTGG
401 CCAAGAGTTA CGGGATTC AATCATGAAA CCTCAGCCAA GACCAGACAG
451 GGTGTTGAAG ATGCTTTT CACACTGGTA AGAGAAATAC GCCAGTACCG
501 AATGAAAAAA CTCAACAGCA GTGATGATGG GACTCAGGGT TGTATGGGAT
551 TGCCATGTGT GGTGATGTA
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Mutations:

NRAS-WT (P1015a): No mutation
 NRAS-S17N (P1015b): AGC to AAC
 NRAS-G12V (P1015c): GGT to GTT

References:

The following GenBank entries are the reference sequences compiled from various individual entries from many laboratories. They have a regularly updated collection of references. The OMIM (#164790) describe the SNPs/variations of the gene and their associations with various genetic diseases.

GenBank Entry: [NM_002524](#)

NCBI Text Ref: [164790](#)

Protein Sequence: [NP_002515](#)