SANTA CRUZ BIOTECHNOLOGY, INC.

GRAMD4 siRNA (m): sc-145754

BACKGROUND

The gene encoding GRAMD4 (GRAM domain-containing protein 4) maps to human chromosome 22, which houses over 500 genes and is the second smallest human chromosome. GRAMD4, also designated death-inducing protein (DIP), is a 578 amino acid mitochondrial membrane protein that acts as an essential mediator of the p53-independent E2F-1 death pathway, which is frequently found to be deregulated in several types of cancers. Overexpression of GRAMD4 results in a strong apoptotic response involving caspase-3 activation and cleavage of poly(ADP-ribose)-polymerase. GRAMD4 is expressed in lung and in primary lung squamous cell carcinoma (LSCC) and shows upregulation in mitochondria by E2F-1 after addition of 4-hydroxytamoxifen. This evidence suggests that GRAMD4 may be a potential target for cancer therapies. There are two isoforms of GRAMD4 which are produced as a result of alternative splicing events.

REFERENCES

1. Gilbert, F. 1998. Disease genes and chromosomes: disease maps of the human genome. Chromosome 22. Genet. Test. 2: 89-97.
2. Schwab, S.G., et al. 1999. Chromosome 22 workshop report. Am. J. Med. Genet. 88: 276-278.
3. Tsilchorozidou, T., et al. 2004. Constitutional rearrangements of chromosome 22 as a cause of neurofibromatosis 2. J. Med. Genet. 41: 529-534.
4. Stanelle, J., et al. 2005. A novel mitochondrial protein DIP mediates E2F1-induced apoptosis independently of p53. Cell Death Differ. 12: 347-357.
5. Arinami, T. 2006. Analyses of the associations between the genes of 22q11 deletion syndrome and schizophrenia. J. Hum. Genet. 51: 1037-1045.
6. Paylor, R., et al. 2006. Tbx1 haploinsufficiency is linked to behavioral disorders in mice and humans: implications for 22q11 deletion syndrome. Proc. Natl. Acad. Sci. USA 103: 7729-7734.
7. Zheng, X., et al. 2006. Bcr and its mutants, the reciprocal t(9;22)-associated ABL/BCR fusion proteins, differentially regulate the cytoskeleton and cell motility. BMC Cancer 6: 262.
8. Hay, B.N. 2007. Deletion 22q11: spectrum of associated disorders. Semin. Pediatr. Neurol. 14: 136-139.

CHROMOSOMAL LOCATION

Genetic locus: Gramd4 (mouse) mapping to 15 E2.

PRODUCT

GRAMD4 siRNA (m) is a pool of 3 target-specific 19-25 nt siRNAs designed to knock down gene expression. Each vial contains 3.3 nmol of lyophilized siRNA, sufficient for a 10 µM solution once resuspended using protocol below. Suitable for 50-100 transfections. Also see GRAMD4 shRNA Plasmid (m): sc-145754-SH and GRAMD4 shRNA (m) Lentiviral Particles: sc-145754-V as alternate gene silencing products.

For independent verification of GRAMD4 (m) gene silencing results, we also provide the individual siRNA duplex components. Each is available as 3.3 nmol of lyophilized siRNA. These include: sc-145754A, sc-145754B and sc-145754C.

STORAGE AND RESUSPENSION

Store lyophilized siRNA duplex at -20° C with desiccant. Stable for at least one year from the date of shipment. Once resuspended, store at -20° C, avoid contact with RNAses and repeated freeze thaw cycles.

Resuspend lyophilized siRNA duplex in 330 µl of the RNAse-free water provided. Resuspension of the siRNA duplex in 330 µl of RNAse-free water makes a 10 µM solution in a 10 µM Tris-HCl, pH 8.0, 20 mM NaCl, 1 mM EDTA buffered solution.

APPLICATIONS

GRAMD4 siRNA (m) is recommended for the inhibition of GRAMD4 expression in mouse cells.

SUPPORT REAGENTS

For optimal siRNA transfection efficiency, Santa Cruz Biotechnology's siRNA Transfection Reagent: sc-29528 (0.3 ml), siRNA Transfection Medium: sc-36868 (20 ml) and siRNA Dilution Buffer: sc-29527 (1.5 ml) are recommended. Control siRNAs or Fluorescein Conjugated Control siRNAs are available as 10 µM in 66 µl. Each contain a scrambled sequence that will not lead to the specific degradation of any known cellular mRNA. Fluorescein Conjugated Control siRNAs include: sc-36869, sc-44239, sc-44240 and sc-44241. Control siRNAs include: sc-37007, sc-44230, sc-44231, sc-44232, sc-44233, sc-44234, sc-44235, sc-44236, sc-44237 and sc-44238.

GENE EXPRESSION MONITORING

GRAMD4 (C-8): sc-515128 is recommended as a control antibody for monitoring of GRAMD4 gene expression knockdown by Western Blotting (starting dilution 1:200, dilution range 1:100-1:1000) or immunofluorescence (starting dilution 1:50, dilution range 1:50-1:500).

To ensure optimal results, the following support reagents are recommended: 1) Western Blotting: use m-IgGκ BP-HRP: sc-516102 or m-IgGκ BP-HRP (Cruz Marker): sc-516102-CM (dilution range: 1:1000-1:10000), Cruz Marker™ Molecular Weight Standards: sc-2035, UltraCruz® Blocking Reagent: sc-516214 and Western Blotting Luminol Reagent: sc-2048. 2) Immunofluorescence: use m-IgGκ BP-FITC: sc-516140 or m-IgGκ BP-PE: sc-516141 (dilution range: 1:50-1:200) with UltraCruz® Mounting Medium: sc-24941 or UltraCruz® Hard-set Mounting Medium: sc-359850.

RT-PCR REAGENTS

Semi-quantitative RT-PCR may be performed to monitor GRAMD4 gene expression knockdown using RT-PCR Primer: GRAMD4 (m)-PR: sc-145754-PR (20 µl). Annealing temperature for the primers should be 55-60° C and the extension temperature should be 68-72° C.

RESEARCH USE

For research use only, not for use in diagnostic procedures.

PROTOCOLS

See our web site at www.scbt.com for detailed protocols and support products.