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K-Ras shRNA (m) Lentiviral Particles: sc-43876-V

BACKGROUND

The mammalian Ras (also designated v-Ha-Ras, Harvey rat sarcoma viral oncogene homolog, HRAS1, K-Ras, N-Ras, RASH1 or c-bas/has) gene family consists of the Harvey and Kirsten Ras genes (c-H-Ras1 and c-K-Ras2), an inactive pseudogene of each (c-H-Ras2 and c-K-Ras1) and the N-Ras gene. The three Ras oncogenes, H-Ras, K-Ras and N-Ras, encode proteins with GTP/GDP binding and GTPase activity. Ras proteins alternate between an inactive form bound to GDP and an active form bound to GTP, activated by a guanine nucleotide-exchange factor (GEF) and inactivated by a GTPase-activating protein (GAP). Ras nomenclature originates from the characterization of human DNA sequences homologous to cloned DNA fragments containing oncogenic sequences of a type C mammalian retrovirus, the Harvey strain of murine sarcoma virus (HaMSV), derived from the rat. Under normal conditions, Ras family members influence cell growth and differentiation events in a subcellular membrane compartmentalization-based signaling system. Oncogenic Ras can deregulate processes that control both cell proliferation and apoptosis. The Ras superfamily of GTP hydrolysis-coupled signal transduction relay proteins can be subclassified into Ras, Rho, Rab and ARF families.

REFERENCES

1. Wong-Staal, F., et al. 1981. Three distinct genes in human DNA related to the transforming genes of mammalian sarcoma retroviruses. *Science* 213: 226-228.
2. Cox, A.D., et al. 2003. The dark side of Ras: regulation of apoptosis. *Oncogene* 22: 8999-9006.
3. Colicelli, J. 2004. Human Ras superfamily proteins and related GTPases. *Sci. STKE* 2004: RE13.

CHROMOSOMAL LOCATION

Genetic locus: Kras (mouse) mapping to 6 G3.

PRODUCT

K-Ras shRNA (m) Lentiviral Particles is a pool of concentrated, transduction-ready viral particles containing 3 target-specific constructs that encode 19-25 nt (plus hairpin) shRNA designed to knock down gene expression. Each vial contains 200 μ l frozen stock containing 1.0×10^6 infectious units of virus (IFU) in Dulbecco's Modified Eagle's Medium with 25 mM HEPES pH 7.3. Suitable for 10-20 transductions. Also see K-Ras siRNA (m): sc-43876 and K-Ras shRNA Plasmid (m): sc-43876-SH as alternate gene silencing products.

STORAGE

Store lentiviral particles at -80°C . Stable for at least one year from the date of shipment. Once thawed, particles can be stored at 4°C for up to one week. Avoid repeated freeze thaw cycles.

PROTOCOLS

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

APPLICATIONS

K-Ras shRNA (m) Lentiviral Particles is recommended for the inhibition of K-Ras expression in mouse cells.

SUPPORT REAGENTS

Control shRNA Lentiviral Particles: sc-108080. Available as 200 μ l frozen viral stock containing 1.0×10^6 infectious units of virus (IFU); contains an shRNA construct encoding a scrambled sequence that will not lead to the specific degradation of any known cellular mRNA.

GENE EXPRESSION MONITORING

K-Ras-2A (C-17): sc-522 is recommended as a control antibody for monitoring of K-Ras 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 (secondary) reagents are recommended: 1) Western Blotting: use goat anti-rabbit IgG-HRP: sc-2004 (dilution range: 1:2000-1:100,000) or Cruz Marker™ compatible goat anti-rabbit IgG-HRP: sc-2030 (dilution range: 1:2000-1:5000), Cruz Marker™ Molecular Weight Standards: sc-2035, TBS Blotto A Blocking Reagent: sc-2333 and Western Blotting Luminol Reagent: sc-2048. 2) Immunofluorescence: use goat anti-rabbit IgG-FITC: sc-2012 (dilution range: 1:100-1:400) or goat anti-rabbit IgG-TR: sc-2780 (dilution range: 1:100-1:400) with UltraCruz™ Mounting Medium: sc-24941.

RT-PCR REAGENTS

Semi-quantitative RT-PCR may be performed to monitor K-Ras gene expression knockdown using RT-PCR Primer: K-Ras (m)-PR: sc-43876-PR (20 μ l, 498 bp). Annealing temperature for the primers should be $55-60^\circ\text{C}$ and the extension temperature should be $68-72^\circ\text{C}$.

BIOSAFETY

Lentiviral particles can be employed in standard Biosafety Level 2 tissue culture facilities (and should be treated with the same level of caution as with any other potentially infectious reagent). Lentiviral particles are replication-incompetent and are designed to self-inactivate after transduction and integration of shRNA constructs into genomic DNA of target cells.

RESEARCH USE

The purchase of this product conveys to the buyer the nontransferable right to use the purchased amount of the product and all replicates and derivatives for research purposes conducted by the buyer in his laboratory only (whether the buyer is an academic or for-profit entity). The buyer cannot sell or otherwise transfer (a) this product (b) its components or (c) materials made using this product or its components to a third party, or otherwise use this product or its components or materials made using this product or its components for Commercial Purposes.