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E2F-7 shRNA (h) Lentiviral Particles: sc-44590-V

BACKGROUND

The human retinoblastoma gene product appears to play an important role in the negative regulation of cell proliferation. Functional inactivation of Rb can be mediated either through mutation or as a consequence of interaction with DNA tumor virus-encoded proteins. Of all the Rb associations described to date, the identification of a complex between Rb and the transcription factor E2F most directly implicates Rb in regulation of cell proliferation. E2F was originally identified through its role in transcriptional activation of the adenovirus E2 promoter. Sequences homologous to the E2F binding site have been found upstream of a number of genes that encode proteins with putative functions in the G₁ and S phases of the cell cycle. E2F-1 is a member of a broader family of transcription regulators including E2F-2, E2F-3, E2F-4, E2F-5, E2F-6 and E2F-7 each of which forms heterodimers with a second protein, DP-1, forming an "active" E2F transcriptional regulatory complex.

REFERENCES

1. Chellappan, S., et al. 1991. The E2F transcription factor is a cellular target for the RB protein. *Cell* 65: 1053-1061.
2. Chittenden, T., et al. 1991. The T/E1A-binding domain of the retinoblastoma product can interact selectively with a sequence-specific DNA-binding protein. *Cell* 65: 1073-1082.
3. Helin, K., et al. 1992. A cDNA encoding a pRB-binding protein with properties of the transcription factor E2F. *Cell* 70: 337-350.
4. Helin, K., et al. 1993. Heterodimerization of the transcription factors E2F-1 and DP-1 leads to cooperative *trans*-activation. *Genes Dev.* 7: 1850-1861.
5. Krek, W., et al. 1993. Binding to DNA and the retinoblastoma gene product promoted by complex formation of different E2F family members. *Science* 262: 1557-1560.

CHROMOSOMAL LOCATION

Genetic locus: E2F7 (human) mapping to 12q21.2.

PRODUCT

E2F-7 shRNA (h) 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 µl frozen stock containing 1.0 x 10⁶ 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 E2F-7 siRNA (h): sc-44590 and E2F-7 shRNA Plasmid (h): sc-44590-SH as alternate gene silencing products.

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.

APPLICATIONS

E2F-7 shRNA (h) Lentiviral Particles is recommended for the inhibition of E2F-7 expression in human cells.

SUPPORT REAGENTS

Control shRNA Lentiviral Particles: sc-108080. Available as 200 µl frozen viral stock containing 1.0 x 10⁶ 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

E2F-7 (N-20): sc-32574 is recommended as a control antibody for monitoring of E2F-7 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 donkey anti-goat IgG-HRP: sc-2020 (dilution range: 1:2000-1:100,000) or Cruz Marker™ compatible donkey anti-goat IgG-HRP: sc-2033 (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 donkey anti-goat IgG-FITC: sc-2024 (dilution range: 1:100-1:400) or donkey anti-goat IgG-TR: sc-2783 (dilution range: 1:100-1:400) with UltraCruz™ Mounting Medium: sc-24941.

RT-PCR REAGENTS

Semi-quantitative RT-PCR may be performed to monitor E2F-7 gene expression knockdown using RT-PCR Primer: E2F-7 (h)-PR: sc-44590-PR (20 µl, 494 bp). Annealing temperature for the primers should be 55-60° C and the extension temperature should be 68-72° 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.

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.