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ATR shRNA (h2) Lentiviral Particles: sc-44284-V

BACKGROUND

Members of the PIK (phosphatidylinositol kinase)-related kinase family are high molecular weight kinases involved in cell cycle progression, DNA recombination and detection of DNA damage. One member of the PI 3-/PI 4-kinase family is ATR (ataxia-telangiectasia- and Rad3-related), also known as FRP1 (for FRAP-related protein 1). ATR is most closely related to ATM, a protein kinase encoded by the gene mutated in ataxia telangiectasia. ATR is also closely related to three of the family members involved in checkpoint function: Mei-41 (*Drosophila*), Mec1p (*S. cerevisiae*) and Rad3 (*Schizosaccharomyces pombe*), and as such may be the functional human counterpart of these proteins. This kinase has been shown to phosphorylate checkpoint kinase CHK1, checkpoint proteins Rad17 and Rad9, as well as tumor suppressor protein BRCA1. In addition, ATR is essential for early embryonic development. The protein encoded by the human ATR gene localizes to intranuclear foci after DNA damage or inhibition of replication.

REFERENCES

1. Cimprich, K., et al. 1996. cDNA cloning and gene mapping of a candidate human cell cycle checkpoint protein. *Proc. Nat. Acad. Sci. USA* 93: 2850-2855.
2. Keegan, K., et al. 1996. The ATR and Atm protein kinases associate with different sites along meiotically pairing chromosomes. *Genes Dev.* 10: 2423-2437.
3. Schmidt, D.R. and Schreiber, S.L. 1999. Molecular association between ATR and two components of the nucleosome remodeling and deacetylating complex, HDAC2 and CHD4. *Biochemistry* 38: 14711-14717.
4. Hall-Jackson, C.A., et al. 1999. ATR is a caffeine-sensitive, DNA-activated protein kinase with a substrate specificity distinct from DNA-PK. *Oncogene* 18: 6707-6713.
5. Wang, Y., et al. 2003. MSH2 and ATR form a signaling module and regulate two branches of the damage response to DNA methylation. *Proc. Natl. Acad. Sci. USA* 100: 15387-15392.

CHROMOSOMAL LOCATION

Genetic locus: ATR (human) mapping to 3q23.

PRODUCT

ATR shRNA (h2) 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 ATR siRNA (h2): sc-44284 and ATR shRNA Plasmid (h2): sc-44284-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.

APPLICATIONS

ATR shRNA (h2) Lentiviral Particles is recommended for the inhibition of ATR expression in human 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

ATR (N-19): sc-1887 is recommended as a control antibody for monitoring of ATR 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 ATR gene expression knockdown using RT-PCR Primer: ATR (h2)-PR: sc-44284-PR (20 μ l, 542 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.

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