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# ATRIP shRNA (h) Lentiviral Particles: sc-44800-V

## BACKGROUND

DNA damage or incomplete replication of DNA results in the inhibition of cell cycle progression at the G<sub>1</sub> to S or the G<sub>2</sub> to M phase transition by conserved regulatory mechanisms known as cell cycle checkpoints. Checkpoint proteins include Rad17, which is involved in regulating cell cycle progression at the G<sub>1</sub> checkpoint as well as Chk1, Chk2, Rad1, Rad9 and Hus1, which are involved in regulating cell cycle arrest at the G<sub>2</sub> checkpoint. In response to DNA damage, ATM and ATR kinases are important for cell cycle checkpoint response signalling. ATR-interacting protein (ATRIP), also designated ATM and Rad3-related-interacting protein, is required for checkpoint signaling after DNA damage. It is also important for ATR expression, which regulates DNA replication and damage checkpoint responses. ATRIP is a ubiquitously expressed protein that can form heterodimers with ATR. After dimerization they bind the RPA complex and are recruited to single stranded DNA. ATRIP is a nuclear protein that may also play a role in protein stabilization.

## REFERENCES

1. Cortez, D., et al. 2001. ATR and ATRIP: partners in checkpoint signaling. *Science* 294: 1713-1716.
2. Zou, L., et al. 2003. Sensing DNA damage through ATRIP recognition of RPA-ssDNA complexes. *Science* 300: 1542-1548.
3. Ball, H.L., et al. 2005. ATRIP oligomerization is required for ATR-dependent checkpoint signaling. *J. Biol. Chem.* 280: 31390-31396.
4. Kim, S.M., et al. 2005. Phosphorylation of Chk1 by ATM- and Rad3-related (ATR) in *Xenopus* egg extracts requires binding of ATRIP to ATR but not the stable DNA-binding. *J. Biol. Chem.* 280: 38355-38364.
5. Itakura, E., et al. 2005. Dimerization of the ATRIP protein through the coiled-coil motif and its implication to the maintenance of stalled replication forks. *Mol. Biol. Cell* 16: 5551-5562.
6. SWISS-PROT/TrEMBL (Q8WXE1). World Wide Web URL: <http://www.expasy.ch/sprot/sprot-top.html>

## CHROMOSOMAL LOCATION

Genetic locus: ATRIP (human) mapping to 3p21.31.

## PRODUCT

ATRIP shRNA (h) Lentiviral Particles are concentrated, transduction-ready viral particles containing a target-specific construct that encodes a 19-25 nt (plus hairpin) shRNA designed to knock down gene expression. Each vial contains 200 µl frozen stock containing 1.0 x 10<sup>6</sup> 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 ATRIP siRNA (h): sc-44800 and ATRIP shRNA Plasmid (h): sc-44800-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

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

## SUPPORT REAGENTS

Control shRNA Lentiviral Particles: sc-108080. Available as 200 µl frozen viral stock containing 1.0 x 10<sup>6</sup> 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

ATRIP (F-7): sc-365383 is recommended as a control antibody for monitoring of ATRIP 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-mouse IgG-HRP: sc-2005 (dilution range: 1:2000-1:32,000) or Cruz Marker™ compatible goat anti-mouse IgG-HRP: sc-2031 (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-mouse IgG-FITC: sc-2010 (dilution range: 1:100-1:400) or goat anti-mouse IgG-TR: sc-2781 (dilution range: 1:100-1:400) with UltraCruz™ Mounting Medium: sc-24941.

## RT-PCR REAGENTS

Semi-quantitative RT-PCR may be performed to monitor ATRIP gene expression knockdown using RT-PCR Primer: ATRIP (h)-PR: sc-44800-PR (20 µl). 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.

## 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.

## PROTOCOLS

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