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SANTA CRUZ BIOTECHNOLOGY, INC.

ATRIP siRNA (m): sc-44801

BACKGROUND

DNA damage or incomplete replication of DNA results in the inhibition of cell cycle progression at the G_1 to S or the G_2 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_1 checkpoint as well as Chk1, Chk2, Rad1, Rad9 and Hus1, which are involved in regulating cell cycle arrest at the G_2 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 (mouse) mapping to 9 F2.

PRODUCT

ATRIP 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 ATRIP shRNA Plasmid (m): sc-44801-SH and ATRIP shRNA (m) Lentiviral Particles: sc-44801-V as alternate gene silencing products.

For independent verification of ATRIP (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-44801A, sc-44801B and sc-44801C.

PROTOCOLS

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

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

ATRIP siRNA (m) is recommended for the inhibition of ATR-Interacting Protein 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

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 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 ATR-Interacting Protein gene expression knockdown using RT-PCR Primer: ATRIP (m)-PR: sc-44801-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.