SANTA CRUZ BIOTECHNOLOGY, INC.

Rad1 siRNA (m): sc-36357

BACKGROUND

DNA damage or incomplete replication of DNA results in inhibition of cell cycle progression at the G_1-S or G_2-M checkpoints by conserved regulatory mechanisms. Rad17 is involved in regulation of cell cycle arrest at the G_1 checkpoint, whereas Chk1, Rad1, Rad9 and Hus1 are involved in regulation of cell cycle arrest at the G_2 checkpoint. Over-expression of Rad17 results in p53 activation and an accumulation of cells in G_1 phase. Chk1 functions as an essential component in the G_2 DNA damage checkpoint by phosphorylating Cdc25C in response to DNA damage, thus inhibiting mitosis. Hus1 and Rad9 exhibit conserved function in fission yeast and higher eukaryotes. Hus1 has been shown to be phosphorylated in response to DNA damage, a process which requires rad checkpoint genes. Rad9 is thought to be a candidate tumor suppressor gene because it is localized to a region of human chromosome 11 containing a number of tumor suppressor loci.

REFERENCES

1. Carr, A.M., et al. 1995. The Chk1 pathway is required to prevent mitosis following cell-cycle arrest at "start". Curr. Biol. 5: 1179-1190.
2. Lieberman, H.B., et al. 1996. A human homolog of the *Schizosaccharomyces pombe* Rad9+ checkpoint control gene. Proc. Natl. Acad. Sci. USA 93: 13890-13895.
3. Sanchez, Y., et al. 1997. Conservation of the Chk1 checkpoint pathway in mammals: linkage of DNA damage to Cdk regulation through Cdc25. Science 277: 1497-1501.
4. O'Connell, M.J., et al. 1997. Chk1 is a Wee1 kinase in the G_2 DNA damage checkpoint inhibiting Cdc2 by Y15 phosphorylation. EMBO J. 16: 545-554.
5. Peng, C.Y., et al. 1997. Mitotic and G_2 checkpoint control: regulation of 14-3-3 protein binding by phosphorylation of Cdc25C on Serine-216. Science 277: 1501-1505.
6. Kostrub, C.F., et al. 1998. Hus1p, a conserved fission yeast checkpoint protein, interacts with Rad1p and is phosphorylated in response to DNA damage. EMBO J. 17: 2055-2066.
7. Bao, S., et al. 1999. HRad17, a human homologue of the *Schizosaccharomyces pombe* checkpoint gene rad17, is overexpressed in colon carcinoma. Cancer Res. 59: 2023-2028.

CHROMOSOMAL LOCATION

Genetic locus: Rad1 (mouse) mapping to 15 A1.

PRODUCT

Rad1 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 Rad1 shRNA Plasmid (m): sc-36357-SH and Rad1 shRNA (m) Lentiviral Particles: sc-36357-V as alternate gene silencing products.

For independent verification of Rad1 (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-36357A, sc-36357B and sc-36357C.

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

Rad1 siRNA (m) is recommended for the inhibition of Rad1 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

Rad1 (D-6): sc-166515 is recommended as a control antibody for monitoring of Rad1 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 Rad1 gene expression knockdown using RT-PCR Primer: Rad1 (m)-PR: sc-36357-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.

PROTOCOLS

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