SANTA CRUZ BIOTECHNOLOGY, INC.

Rad9 siRNA (h): sc-36364

BACKGROUND

DNA damage or incomplete replication of DNA results in the inhibition of cell cycle progression at the G_1 to S or G_2 to M phase checkpoints by conserved regulatory mechanisms. Chk1, Rad9 and Hus1 are involved in the signal transduction cascade that regulates cell cycle arrest at the G_2 checkpoint. Chk1 functions as an essential component in the G_2 phase DNA damage checkpoint, as it phosphorylates Cdc25C in response to DNA damage and thereby inhibits mitosis. Two related mammalian proteins, Hus1 and Rad9, share conserved sequence identity and function to the yeast homologs of the same names. *In vivo,* Rad9 is highly phosphorylated and directly associates with two other checkpoint control proteins, Rad1 and Hus1. Additionally, Rad9 associates with anti-apoptotic Bcl-2 family proteins Bcl-2 and Bcl-x_L, but not with the pro-apoptotic Bax and Bad proteins. Overexpression of Rad9 induces apoptosis and indicates that Rad9 may have an additional role in regulating apoptosis after DNA damage.

REFERENCES

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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. St. Onge, R.P., et al. 1999. The human G2 checkpoint control protein hRAD9 is a nuclear phosphoprotein that forms complexes with hRAD1 and hHUS1. Mol. Biol. Cell 10: 1985-1995.
8. Komatsu, K., et al. 2000. Human homologue of *S. pombe* rad9 interacts with Bcl-2/Bcl-x_L and promotes apoptosis. Nat. Cell Biol. 2: 1-6.

CHROMOSOMAL LOCATION

Genetic locus: RAD9A (human) mapping to 11q13.2.

PRODUCT

Rad9 siRNA (h) is a target-specific 19-25 nt siRNA 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 Rad9 shRNA Plasmid (h): sc-36364-SH and Rad9 shRNA (h) Lentiviral Particles: sc-36364-V as alternate gene silencing 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

Rad9 siRNA (h) is recommended for the inhibition of Rad9 expression in human 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

Rad9 (B-8): sc-74464 is recommended as a control antibody for monitoring of Rad9 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 Rad9 gene expression knockdown using RT-PCR Primer: Rad9 (h)-PR: sc-36364-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.