

Rad9B siRNA (h): sc-95745

BACKGROUND

DNA damage or incomplete replication of DNA results in the inhibition of cell cycle progression at the G₁ to S or G₂ to M phase checkpoints by conserved regulatory mechanisms. Rad9B (RAD9 homolog B) is a 426 amino acid cell cycle checkpoint control protein that is expressed in testis and skeletal muscle. Belonging to the Rad9 family, Rad9B interacts with Hus1, Hus1B, Rad1, Rad9 and Rad17. Rad9B and Rad9 share extensive amino acid homology throughout their entire sequences, suggesting similar biochemical reactions. 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. Rad9B exists as five alternatively spliced isoforms that are encoded by a gene located on human chromosome 12q24.11.

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. 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.
3. Kostub, 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.
4. 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.
5. 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.
6. Dufault, V.M., et al. 2003. Identification and characterization of RAD9B, a paralog of the RAD9 checkpoint gene. *Genomics* 82: 644-651.
7. Online Mendelian Inheritance in Man, OMIM[™]. 2003. Johns Hopkins University, Baltimore, MD. MIM Number: 608368. World Wide Web URL: <http://www.ncbi.nlm.nih.gov/omim/>
8. Abdu, U., et al. 2007. An essential role for *Drosophila* hus1 in somatic and meiotic DNA damage responses. *J. Cell Sci.* 120: 1042-1049.
9. Sierant, M.L., et al. 2010. The Rad9A checkpoint protein is required for nuclear localization of the claspin adaptor protein. *Cell Cycle* 9: 548-556.

CHROMOSOMAL LOCATION

Genetic locus: RAD9B (human) mapping to 12q24.11.

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.

PRODUCT

Rad9B siRNA (h) 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 μ M solution once resuspended using protocol below. Suitable for 50-100 transfections. Also see Rad9B shRNA Plasmid (h): sc-95745-SH and Rad9B shRNA (h) Lentiviral Particles: sc-95745-V as alternate gene silencing products.

For independent verification of Rad9B (h) gene silencing results, we also provide the individual siRNA duplex components. Each is available as 3.3 nmol of lyophilized siRNA. These include: sc-95745A, sc-95745B and sc-95745C.

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 μ l of the RNase-free water provided. Resuspension of the siRNA duplex in 330 μ l of RNase-free water makes a 10 μ M solution in a 10 μ M Tris-HCl, pH 8.0, 20 mM NaCl, 1 mM EDTA buffered solution.

APPLICATIONS

Rad9B siRNA (h) is recommended for the inhibition of Rad9B 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 μ M in 66 μ l. 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.

RT-PCR REAGENTS

Semi-quantitative RT-PCR may be performed to monitor Rad9B gene expression knockdown using RT-PCR Primer: Rad9B (h)-PR: sc-95745-PR (20 μ l). Annealing temperature for the primers should be 55-60° C and the extension temperature should be 68-72° C.