

Rad23B siRNA (h): sc-60812

BACKGROUND

Mammalian cells express two Rad23 (genome repair protein) homologs: Rad23A (also designated HR23A) and Rad23B (also designated HR23B). In typical cells, mouse Rad23B is approximately ten times more abundant than mouse Rad23A. Endogenous XPC (xeroderma pigmentosum C protein) located in wildtype mouse embryonic fibroblasts is relatively stable; its steady-state level and stability appear to be significantly reduced by a targeted interruption of the mouse Rad23B gene, but not by that of mouse Rad23A. Loss of both mouse Rad23 genes causes a strong further reduction of the XPC protein level. The RAD23 genes (RAD23A and RAD23B), which encode the human Rad23 proteins, are crucial for excision-repair of UV-damaged DNA. RAD23 genes resemble the other DNA repair genes, RAD2, RAD6, RAD7, RAD18 and RAD54, all of which also exhibit increased transcription in response to DNA damage and during meiosis. Rad23 is a nuclear protein containing a ubiquitin-like domain required for biological functions. The protein, which is highly conserved, is involved in nucleotide excision repair (NER) that associates with the proteasome via its N-terminus. The C-terminal ubiquitin-associated domain of Rad23 is evolutionarily conserved from yeast to humans. Rad23 may also act as a regulator for the activity of the 26S Proteasome.

REFERENCES

1. Elder, R.T., et al. 2002. Involvement of rhp23, a *Schizosaccharomyces pombe* homolog of the human hHR23A and *Saccharomyces cerevisiae* Rad23 nucleotide excision repair genes, in cell cycle control and protein ubiquitination. *Nucleic Acids Res.* 30: 581-591.
2. Ng, J.M., et al. 2003. A novel regulation mechanism of DNA repair by damage-induced and Rad23-dependent stabilization of xeroderma pigmentosum group C protein. *Genes Dev.* 17: 1630-1645.
3. Wang, Q., et al. 2003. Ubiquitin recognition by the DNA repair protein hHR23A. *Biochemistry* 42: 13529-13535.
4. Kamionka, M. and Feigon, J. 2004. Structure of the XPC binding domain of hHR23A reveals hydrophobic patches for protein interaction. *Protein Sci.* 13: 2370-2377.
5. Okuda, Y., et al. 2004. Relative levels of the two mammalian Rad23 homologs determine composition and stability of the xeroderma pigmentosum group C protein complex. *DNA Repair* 3: 1285-1295.

CHROMOSOMAL LOCATION

Genetic locus: RAD23B (human) mapping to 9q31.2.

PRODUCT

Rad23B 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 Rad23B shRNA Plasmid (h): sc-60812-SH and Rad23B shRNA (h) Lentiviral Particles: sc-60812-V as alternate gene silencing products.

For independent verification of Rad23B (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-60812A, sc-60812B and sc-60812C.

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

Rad23B siRNA (h) is recommended for the inhibition of Rad23B 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.

GENE EXPRESSION MONITORING

Rad23B (H-8): sc-137088 is recommended as a control antibody for monitoring of Rad23B 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-IgG κ BP-HRP: sc-516102 or m-IgG κ 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-IgG κ BP-FITC: sc-516140 or m-IgG κ 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 Rad23B gene expression knockdown using RT-PCR Primer: Rad23B (h)-PR: sc-60812-PR (20 μ l, 423 bp). 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.