

DDX8 siRNA (h): sc-93820

BACKGROUND

DEAD-box proteins, characterized by the conserved motif Asp-Glu-Ala-Asp, are putative RNA helicases implicated in several cellular processes involving modifications of RNA secondary structure and ribosome/spliceosome assembly. Based on their distribution patterns, some members of this family may be involved in embryogenesis, spermatogenesis, and cellular growth and division. DDX8 (DEAH-box polypeptide 8), also known as DHX8, HRH1 or PRP22, contains an arginine- and serine-rich domain (RS domain) that is characteristic of some splicing factors. DDX8 may be targeted to the spliceosome through an interaction involving its RS domain.

REFERENCES

1. Ono, Y., et al. 1994. Identification of a putative RNA helicase (HRH1), a human homolog of yeast Prp22. *Mol. Cell. Biol.* 14: 7611-7620.
2. Py, B., et al. 1996. A DEAD-box RNA helicase in the *Escherichia coli* RNA degradosome. *Nature* 381: 169-172.
3. Eisen, A., et al. 1998. A novel DEAD-box RNA helicase exhibits high sequence conservation from yeast to humans. *Biochim. Biophys. Acta* 1397: 131-136.
4. Kittler, R., et al. 2004. An endoribonuclease-prepared siRNA screen in human cells identifies genes essential for cell division. *Nature* 432: 1036-1040.
5. Zhang, D.Y., et al. 2006. Molecular cloning and characterization of a putative nuclear DEAD box RNA helicase in the spruce budworm, *Choristoneura fumiferana*. *Arch. Insect Biochem. Physiol.* 61: 209-219.
6. Jain, C. 2008. The *E. coli* RhlE RNA helicase regulates the function of related RNA helicases during ribosome assembly. *RNA* 14: 381-389.

CHROMOSOMAL LOCATION

Genetic locus: DHX8 (human) mapping to 17q21.31.

PRODUCT

DDX8 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 DDX8 shRNA Plasmid (h): sc-93820-SH and DDX8 shRNA (h) Lentiviral Particles: sc-93820-V as alternate gene silencing products.

For independent verification of DDX8 (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-93820A, sc-93820B and sc-93820C.

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.

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

DDX8 siRNA (h) is recommended for the inhibition of DDX8 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

DDX8 (F-1): sc-515533 is recommended as a control antibody for monitoring of DDX8 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 DDX8 gene expression knockdown using RT-PCR Primer: DDX8 (h)-PR: sc-93820-PR (20 μ l, 576 bp). Annealing temperature for the primers should be 55-60° C and the extension temperature should be 68-72° C.