SANTA CRUZ BIOTECHNOLOGY, INC.

DDX32 siRNA (h): sc-77143

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. DDX32 (DEAH box polypeptide 32), also known as DHLP1 or DHX32, is a 743 amino acid nuclear protein that localizes to the mitochondria and is a member of the DEAD box helicase family. Expressed in various tissues, DDX32 is up-regulated by Ionomycin in T lymphocytes and down-regulated in acute lymphoblastic leukemia. Considered a novel RNA helicase, DDX32 may play an important role in the development of colorectal cancer and may be involved in regulating T-cell response to certain apoptotic stimuli.

REFERENCES

1. Abdelhaleem, M. 2002. The novel helicase homologue DDX32 is down-regulated in acute lymphoblastic leukemia. Leuk. Res. 26: 945-954.
2. Abdelhaleem, M., et al. 2005. DHX32 expression suggests a role in lymphocyte differentiation. Anticancer Res. 25: 2645-2648.
3. Alli, Z., et al. 2005. The activation-induced expression of DHX32 in Jurkat T cells is specific and involves calcium and nuclear factor of activated T cells. Cell. Immunol. 237: 141-146.
4. Alli, Z., et al. 2005. Expression of DHX32 in lymphoid tissues. Exp. Mol. Pathol. 79: 219-223.
5. Alli, Z., et al. 2006. Nuclear and mitochondrial localization of the putative RNA helicase DHX32. Exp. Mol. Pathol. 81: 245-248.
6. Alli, Z., et al. 2007. A role for DHX32 in regulating T cell apoptosis. Anticancer Res. 27: 373-377.
7. Chen, Y., et al. 2007. Altered distribution of heat shock protein 60 (HSP 60) with dysregulated expression of DHX32. Exp. Mol. Pathol. 82: 256-261.

CHROMOSOMAL LOCATION

Genetic locus: DHX32 (human) mapping to 10q26.2.

PRODUCT

DDX32 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 µM solution once resuspended using protocol below. Suitable for 50-100 transfections. Also see DDX32 shRNA Plasmid (h): sc-77143-SH and DDX32 shRNA (h) Lentiviral Particles: sc-77143-V as alternate gene silencing products.

For independent verification of DDX32 (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-77143A, sc-77143B and sc-77143C.

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 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

DDX32 siRNA (h) is recommended for the inhibition of DDX32 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

DDX32 (H-3): sc-398743 is recommended as a control antibody for monitoring of DDX32 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 DDX32 gene expression knockdown using RT-PCR Primer: DDX32 (h)-PR: sc-77143-PR (20 µl, 560 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.