SANTA CRUZ BIOTECHNOLOGY, INC.

DDX3X siRNA (m): sc-77109

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 and spliceosome assembly. Based on their distribution patterns, some members of this family may be involved in embryogenesis, spermatogenesis, and cellular growth and division. DDX3 (DEAD box polypeptide 3) is involved in RNA metabolism. Two DDX3 paralogs are found in humans; DDX3X is encoded by a gene found on the X chromosome while DDX3Y is encoded by a gene on the Y chromosome. DDX3Y is exclusively expressed in testis and is required for normal spermatogenesis. DDX3X is ubiquitously expressed and predominantly localizes to the nuclear speckles, participating in RNA splicing, transcription, translation initiation, mRNA transport and cell cycle regulation. DDX3X also partakes in HIV-1 replication and hepatitis C viral infections.

REFERENCES

1. Chao, C.H., et al. 2006. DDX3, a DEAD box RNA helicase with tumor growth-suppressive property and transcriptional regulation activity of the $p21^{waf1/cip1}$ promoter, is a candidate tumor suppressor. Cancer Res. 66: 6579-6588.
2. Nekhai, S. and Jeang, K.T. 2006. Transcriptional and post-transcriptional regulation of HIV-1 gene expression: role of cellular factors for Tat and Rev. Future Microbiol. 1: 417-426.
3. Rosner, A. and Rinkevich, B. 2007. The DDX3 subfamily of the DEAD box helicases: divergent roles as unveiled by studying different organisms and *in vitro* assays. Curr. Med. Chem. 14: 2517-2525.
4. Ariumi, Y., et al. 2007. DDX3 DEAD-box RNA helicase is required for hepatitis C virus RNA replication. J. Virol. 81: 13922-13926.
5. Högbom, M., et al. 2007. Crystal structure of conserved domains 1 and 2 of the human DEAD-box helicase DDX3X in complex with the mononucleotide AMP. J. Mol. Biol. 372: 150-159.
6. Rodamilans, B. and Montoya, G. 2007. Expression, purification, crystallization and preliminary X-ray diffraction analysis of the DDX3 RNA helicase domain. Acta Crystallogr. Sect. F Struct. Biol. Cryst. Commun. 63: 283-286.

CHROMOSOMAL LOCATION

Genetic locus: Ddx3x (mouse) mapping to X A1.1.

PRODUCT

DDX3X siRNA (m) 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 DDX3X shRNA Plasmid (m): sc-77109-SH and DDX3X shRNA (m) Lentiviral Particles: sc-77109-V as alternate gene silencing products.

For independent verification of DDX3X (m) gene silencing results, we also provide the individual siRNA duplex components. Each is available as 3.3 nmol of lyophilized siRNA. These include: sc-77109A, sc-77109B and sc-77109C.

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

DDX3X siRNA (m) is recommended for the inhibition of DDX3X expression in mouse 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

DDX3 (2253C5a): sc-81247 is recommended as a control antibody for monitoring of DDX3X 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) Immunoprecipitation: use Protein A/G PLUS-Agarose: sc-2003 (0.5 ml agarose/2.0 ml).

RT-PCR REAGENTS

Semi-quantitative RT-PCR may be performed to monitor DDX3X gene expression knockdown using RT-PCR Primer: DDX3X (m)-PR: sc-77109-PR (20 µl). Annealing temperature for the primers should be 55-60° C and the extension temperature should be 68-72° C.

SELECT PRODUCT CITATIONS

1. Accornero, F., et al. 2017. BEX1 is an RNA-dependent mediator of cardiomyopathy. Nat. Commun. 8: 1875.

RESEARCH USE

For research use only, not for use in diagnostic procedures.