

NOBOX siRNA (h): sc-89594

BACKGROUND

Early ovarian folliculogenesis is characterized by the breakdown of germ cell clusters and formation of primordial follicles. The cessation of ovarian function under the age of 40 years results in premature ovarian failure (POF) and is accompanied by amenorrhea, hypoestrogenism and elevated serum gonadotropin concentrations. 1% of all women are affected by POF, and mutations in a few genes, including inhibin α , fsh receptor and the LH/choriogonadotropin receptor have been linked to POF. In addition, several germ cell specific genes and downstream transcription factors are thought to play an important role in human oogenesis. NOBOX (newborn ovary homeobox gene), an oocyte-specific homeobox gene, is a critical protein involved in early folliculogenesis. Missense mutations have been shown to cause nonsyndromic ovarian failure by disrupting the NOBOX proteins ability to bind to NOBOX DNA-binding element (NBE), and thereby inhibiting its regulation of Pou5f1 and GDF-9. NOBOX expression in the ovary is oocyte specific, but it shows expression in adult testis and pancreas as well.

REFERENCES

1. Suzumori, N., et al. 2002. Nobox is a homeobox-encoding gene preferentially expressed in primordial and growing oocytes. *Mech. Dev.* 111: 137-141.
2. Rajkovic, A., et al. 2004. NOBOX deficiency disrupts early folliculogenesis and oocyte-specific gene expression. *Science* 305: 1157-1159.
3. Zhao, X.X., et al. 2005. Mutational analysis of the homeobox region of the human NOBOX gene in Japanese women who exhibit premature ovarian failure. *Fertil. Steril.* 83: 1843-1844.
4. Choi, Y., et al. 2006. Genetics of early mammalian folliculogenesis. *Cell. Mol. Life Sci.* 63: 579-590.
5. Choi, Y., et al. 2006. Characterization of NOBOX DNA binding specificity and its regulation of Gdf9 and Pou5f1 promoters. *J. Biol. Chem.* 281: 35747-35756.
6. Huntriss, J., et al. 2006. cDNA cloning and expression of the human NOBOX gene in oocytes and ovarian follicles. *Mol. Hum. Reprod.* 12: 283-289.
7. Qin, Y., et al. 2007. NOBOX homeobox mutation causes premature ovarian failure. *Am. J. Hum. Genet.* 81: 576-581.

CHROMOSOMAL LOCATION

Genetic locus: NOBOX (human) mapping to 7q35.

PRODUCT

NOBOX 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 NOBOX shRNA Plasmid (h): sc-89594-SH and NOBOX shRNA (h) Lentiviral Particles: sc-89594-V as alternate gene silencing products.

For independent verification of NOBOX (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-89594A, sc-89594B and sc-89594C.

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

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

NOBOX (A-5): sc-514178 is recommended as a control antibody for monitoring of NOBOX 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 NOBOX gene expression knockdown using RT-PCR Primer: NOBOX (h)-PR: sc-89594-PR (20 μ l). 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.