SANTA CRUZ BIOTECHNOLOGY, INC.

GBX2 siRNA (h): sc-38665

BACKGROUND

The isthmic organizer signals at the mid/hindbrain boundary (MHB) regulate the development and differentiation of the vertebrate caudal midbrain and the anterior hindbrain. The MHB forms at the boundary of expression between homeobox genes GBX2 and OTX2. GBX2 and OTX2 play distinct, essential roles in MHB positioning and development. During development, the GBX2 gene is expressed in the anterior hindbrain. Specifically, GBX2 negatively regulates OTX2 expression along the anterior-posterior axis; GBX2$^-$ mutants demonstrate an expanded OTX2 domain. During development, the GBX2 gene is expressed in the anterior hindbrain. GBX2 is expressed in the adult brain, spleen and female genital tract. The GBX2 gene is overexpressed in human prostate cancer cell lines (TSU-prl, PC3, DU145 and LNCaP). Furthermore, downregulation of GBX2 expression restricts tumorigenicity in human prostate cancer cell lines, which suggests that GBX2 expression may be required for growth of malignant prostate cells.

REFERENCES

1. Bouillet, P., et al. 1995. Sequence and expression pattern of the Stra7 (GBX2) homeobox-containing gene induced by retinoic acid in P19 embryonal carcinoma cells. Dev. Dyn. 204: 372-382.
2. Gao, A.C. et al. 1996. Expression of homeobox gene-GBX2 in human prostatic cancer cells. Prostate 29: 395-398.
3. Gao, A.C., et al. 1998. Downregulation of homeobox gene GBX2 expression inhibits human prostate cancer clonogenic ability and tumorigenicity. Cancer Res. 58: 1391-1394.
4. Millet, S., et al. 1999. A role for GBX2 in repression of OTX2 and positioning the mid/hindbrain organizer. Nature 401: 161-164.
5. Gao, A.C., et al. 2000. Enhanced GBX2 expression stimulates growth of human prostate cancer cells via transcriptional upregulation of the Interleukin 6 gene. Clin. Cancer Res. 6: 493-497.
6. Online Mendelian Inheritance in Man, OMIM™. 2000. Johns Hopkins University, Baltimore, MD. MIM Number: 601135. World Wide Web URL: http://www.ncbi.nlm.nih.gov/omim/
7. Tour, E., et al. 2002. OTX2 can activate the isthmic organizer genetic network in the *Xenopus* embryo. Mech. Dev. 110: 3-13.

CHROMOSOMAL LOCATION

Genetic locus: GBX2 (human) mapping to 2q37.2.

PRODUCT

GBX2 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 GBX2 shRNA Plasmid (h): sc-38665-SH and GBX2 shRNA (h) Lentiviral Particles: sc-38665-V as alternate gene silencing products.

For independent verification of GBX2 (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-38665A, sc-38665B and sc-38665C.

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

GBX2 siRNA (h) is recommended for the inhibition of GBX2 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

GBX2 (BB-9): sc-81963 is recommended as a control antibody for monitoring of GBX2 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 GBX2 gene expression knockdown using RT-PCR Primer: GBX2 (h)-PR: sc-38665-PR (20 µl). 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.