

TFIIB90 siRNA (h): sc-38535

BACKGROUND

RNA polymerase (pol) III synthesizes tRNA, 5s rRNA, 7SL RNA and U6 snRNA and is overexpressed in many transformed cell lines and tumors *in vivo*, since cells must duplicate its protein components before division. Therefore, in order to maintain rapid growth, cells must produce a high level of Pol III transcribed RNA, which requires the presence of the TFIIB and TFIIC2 transcription factor complexes. The TFIIC2 complex is composed of five subunits, TFIIC220, TFIIC110, TFIIC102, TFIIC90 and TFIIC63, that are overexpressed in adenovirus transformed cells as well as in malignant cells *in vivo*, such as ovarian carcinomas. TFIIC2 recruits RNA pol III and TFIIB to promoter elements and may be a key component in the deregulation of malignant cells. The TFIIB complex includes the TATA-binding protein (TBP), TFIIB-related factor 1 (BRF1) and TFIIB", the expression of which are also upregulated in transformed cells. In many carcinomas, the tumor suppressors retinoblastoma (RB) and p53 are inactivated, which affects their ability to bind and inactivate the function of TFIIB.

REFERENCES

1. Scott, M.R., et al. 1983. Activation of mouse genes in transformed cells. *Cell* 34: 557-567.
2. Chen, W., et al. 1997. Expression of neural BC1 RNA: induction in murine tumours. *Eur. J. Cancer* 33: 288-292.
3. Hsieh, Y.J., et al. 1999. The TFIIC90 subunit of TFIIC interacts with multiple components of the RNA polymerase III machinery and contains a histone-specific acetyltransferase activity. *Mol. Cell. Biol.* 19: 7697-7704.
4. Winter, A.G., et al. 2000. RNA polymerase III transcription factor TFIIC2 is overexpressed in ovarian tumors. *Proc. Natl. Acad. Sci. USA* 97: 12619-12624.
5. Moir, R.D., et al. 2000. Interactions between the tetratricopeptide repeat-containing transcription factor TFIIC131 and its ligand, TFIIB70. Evidence for a conformational change in the complex. *J. Biol. Chem.* 275: 26591-26598.
6. McCulloch, V., et al. 2000. Alternatively spliced hBRF variants function at different RNA polymerase III promoters. *EMBO J.* 19: 4134-4143.

CHROMOSOMAL LOCATION

Genetic locus: BRF1 (human) mapping to 14q32.33.

PRODUCT

TFIIB90 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 TFIIB90 shRNA Plasmid (h): sc-38535-SH and TFIIB90 shRNA (h) Lentiviral Particles: sc-38535-V as alternate gene silencing products.

For independent verification of TFIIB90 (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-38535A, sc-38535B and sc-38535C.

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

TFIIB90 siRNA (h) is recommended for the inhibition of TFIIB90-1 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

TFIIB90-1/2/3/5 (A-8): sc-390821 is recommended as a control antibody for monitoring of TFIIB90 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 TFIIB90 gene expression knockdown using RT-PCR Primer: TFIIB90 (h)-PR: sc-38535-PR (20 μ l, 583 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.

PROTOCOLS

See our web site at www.scbt.com for detailed protocols and support products.