

RFX-B siRNA (h): sc-37751

BACKGROUND

EP is a regulatory enhancer element found in several promoters on viral genes, and similar sites are also present in cellular genes, including the MIF-1 binding site (MIE) of the human c-Myc gene, the X box of MHC class II promoters and a binding site in the proliferating cell nuclear antigen promoter. The EP sites present in the X box of MHC class II promoters are distinctly nonpalindromic sequences that contain only a single EP-homologous half-site. The EP element is bound by a ubiquitous nuclear protein complex that consists of homo- and heterodimers involving the RFX1, RFX2 and RFX3 proteins. The RFX proteins represent an essential class II transcription factor family that shares several conserved regions, including the centrally located DNA-binding domain (DBD) and the D region found in the C-terminal part of these proteins which facilitates dimerization. RFX complexes can activate the enhancer elements of several HBV genes and also promote the induction of MHC class II genes in response to interferon- γ stimulation. Two additional subunits, RFX5, RFX-B/Ank, are also involved in the RFX complexes, yet they bind additional elements in the X1 half of the X box.

REFERENCES

1. Dikstein, R., et al. 1990. Functional organization of the hepatitis B virus enhancer. *Mol. Cell. Biol.* 10: 3682-3689.
2. Fontes, J.D., et al. 1997. Assembly of functional regulatory complexes on MHC class II promoters *in vivo*. *J. Mol. Biol.* 270: 336-345.
3. Katan, Y., et al. 1997. The transcriptional activation and repression domains of RFX1, a context-dependent regulator, can mutually neutralize their activities. *Nucleic Acids Res.* 25: 3621-3628.
4. Katan-Khaykovich, Y., et al. 1998. RFX1, a single DNA-binding protein with a split dimerization domain, generates alternative complexes. *J. Biol. Chem.* 273: 24504-24512.
5. Masternak, K., et al. 1998. A gene encoding a novel RFX-associated trans-activator is mutated in the majority of MHC class II deficiency patients. *Nat. Genet.* 20: 273-277.
6. Westerheide, S.D., et al. 1999. Orientation and positional mapping of the subunits of the multicomponent transcription factors RFX and X2BP to the major histocompatibility complex class II transcriptional enhancer. *Nucleic Acids Res.* 27: 1635-1641.

CHROMOSOMAL LOCATION

Genetic locus: RFXANK (human) mapping to 19p13.11.

PRODUCT

RFX-B 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 RFX-B shRNA Plasmid (h): sc-37751-SH and RFX-B shRNA (h) Lentiviral Particles: sc-37751-V as alternate gene silencing products.

For independent verification of RFX-B (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-37751A, sc-37751B and sc-37751C.

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

RFX-B siRNA (h) is recommended for the inhibition of RFX-B 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

RFX-B (E-4): sc-514873 is recommended as a control antibody for monitoring of RFX-B 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 RFX-B gene expression knockdown using RT-PCR Primer: RFX-B (h)-PR: sc-37751-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.