

RBP-J κ siRNA (r): sc-270318

BACKGROUND

Recombination signal binding protein J κ (RBP-J κ), also designated KBF2 or CBF1, is the mammalian homolog of the *Drosophila* suppressor of hairless [Su(H)], a protein involved in the development of the peripheral nervous system. RBP-J κ is ubiquitously expressed in mammalian tissues and is involved in the regulation of gene expression. RBP-J κ has been shown to directly interact with the intercellular domain of the cell surface receptor Notch 1. Proteolytically cleaved Notch 1 translocates to the nucleus, where it binds DNA-bound RBP-J κ and activates transcription of target genes. These genes include NF κ B p52 and the Epstein-Barr virus (EBV) protein EBNA-2, both of which contain RBP-J κ -binding sequences within their promoter regions.

REFERENCES

1. Amakawa, R., et al. 1993. Human J κ recombination signal binding protein gene (IGKJRB): comparison with its mouse homologue. *Genomics* 17: 306-315.
2. Waltzer, L., et al. 1994. The human J κ recombination signal sequence binding protein (RBP-J κ) targets the Epstein-Barr virus EBNA2 protein to its DNA responsive elements. *EMBO J.* 13: 5633-5638.
3. Oka, C., et al. 1995. Disruption of the mouse RBP-J κ gene results in early embryonic death. *Development* 121: 3291-3301.
4. Waltzer, L., et al. 1995. RBP-J κ repression activity is mediated by a corepressor and antagonized by the Epstein-Barr virus transcription factor EBNA2. *Nucleic Acids Res.* 23: 4939-4945.
5. Tamura, K., et al. 1995. Physical interaction between a novel domain of the receptor Notch and the transcription factor RBP-J κ /Su(H). *Curr. Biol.* 5: 1416-1423.
6. Hsieh, J.J., et al. 1996. Truncated mammalian Notch1 activates CBF1/RBP-J κ repressed genes by a mechanism resembling that of Epstein-Barr virus EBNA2. *Mol. Cell. Biol.* 16: 952-959.
7. Oswald, F., et al. 1998. NF κ B2 is a putative target gene of activated Notch-1 via RBP-J κ . *Mol. Cell. Biol.* 18: 2077-2088.

CHROMOSOMAL LOCATION

Genetic locus: Rbpj (rat) mapping to 14q11.

PRODUCT

RBP-J κ siRNA (r) 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 RBP-J κ shRNA Plasmid (r): sc-270318-SH and RBP-J κ shRNA (r) Lentiviral Particles: sc-270318-V as alternate gene silencing products.

For independent verification of RBP-J κ (r) gene silencing results, we also provide the individual siRNA duplex components. Each is available as 3.3 nmol of lyophilized siRNA. These include: sc-270318A, sc-270318B and sc-270318C.

RESEARCH USE

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

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

RBP-J κ siRNA (r) is recommended for the inhibition of RBP-J κ expression in rat 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

RBP-J κ (E-7): sc-271128 is recommended as a control antibody for monitoring of RBP-J κ 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 RBP-J κ gene expression knockdown using RT-PCR Primer: RBP-J κ (r)-PR: sc-270318-PR (20 μ l). Annealing temperature for the primers should be 55-60° C and the extension temperature should be 68-72° C.

PROTOCOLS

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