



E2F-5 siRNA (h): sc-35250

BACKGROUND

The human retinoblastoma gene product appears to play an important role in the negative regulation of cell proliferation. Functional inactivation of Rb can be mediated either through mutation or as a consequence of interaction with DNA tumor virus-encoded proteins. Of all the Rb associations described to date, the identification of a complex between Rb and the transcription factor E2F most directly implicates Rb in regulation of cell proliferation. E2F was originally identified through its role in transcriptional activation of the adenovirus E2 promoter. Sequences homologous to the E2F binding site have been found upstream of a number of genes that encode proteins with putative functions in the G₁ and S phases of the cell cycle. E2F-1 is a member of a broader family of transcription regulators including E2F-2, E2F-3, E2F-4, E2F-5, E2F-6 and E2F-7 each of which forms heterodimers with a second protein, DP-1, forming an "active" E2F transcriptional regulatory complex.

REFERENCES

1. Chellappan, S., et al. 1991. The E2F transcription factor is a cellular target for the RB protein. *Cell* 65: 1053-1061.
2. Chittenden, T., et al. 1991. The T/E1A-binding domain of the retinoblastoma product can interact selectively with a sequence-specific DNA-binding protein. *Cell* 65: 1073-1082.
3. Helin, K., et al. 1992. A cDNA encoding a pRB-binding protein with properties of the transcription factor E2F. *Cell* 70: 337-350.
4. Helin, K., et al. 1993. Heterodimerization of the transcription factors E2F-1 and DP-1 leads to cooperative transactivation. *Genes Dev.* 7: 1850-1861.
5. Krek, W., et al. 1993. Binding to DNA and the retinoblastoma gene product promoted by complex formation of different E2F family members. *Science* 262: 1557-1560.
6. Ginsberg, D., et al. 1994. E2F-4, a new member of the E2F transcription factor family, interacts with p107. *Genes Dev.* 8: 2665-2679.
7. Beijersbergen, R.L., et al. 1994. E2F-4, a new member of the E2F gene family, has oncogenic activity and associates with p107 *in vivo*. *Genes Dev.* 8: 2680-2690.

CHROMOSOMAL LOCATION

Genetic locus: E2F5 (human) mapping to 8q21.2.

PRODUCT

E2F-5 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 E2F-5 shRNA Plasmid (h): sc-35250-SH and E2F-5 shRNA (h) Lentiviral Particles: sc-35250-V as alternate gene silencing products.

For independent verification of E2F-5 (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-35250A, sc-35250B and sc-35250C.

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

E2F-5 siRNA (h) is recommended for the inhibition of E2F-5 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

E2F-5 (C-8): sc-374268 is recommended as a control antibody for monitoring of E2F-5 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 E2F-5 gene expression knockdown using RT-PCR Primer: E2F-5 (h)-PR: sc-35250-PR (20 μ l, 464 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.