

p130 siRNA (m): sc-29426

BACKGROUND

The human retinoblastoma gene product Rb plays an important role in the negative regulation of cell proliferation. The Rb family includes p107 and p130, which form complexes with E2F proteins, and share a high degree of structural homology in the adenovirus E1A binding domain (i.e., "pocket region"), which plays a primary role in the function of these proteins. The Rb family members undergo cell cycle dependent phosphorylation during mid-G₁ to S phase transition, which is dependent upon the activity of cyclin D/cdk4. In contrast to pRb and p107, p130 is also phosphorylated during G₀ and the early G₁ phase of the cell cycle. p130 is specifically phosphorylated on serine and threonine residues in cells arrested in G₀ by serum deprivation or density arrest, and these residues are clustered within a short co-linear region unique to p130 defined as the Loop.

REFERENCES

1. Kovesdi, I., et al. 1986. Identification of a cellular transcription factor involved in E1A transactivation. *Cell* 45: 219-228.
2. Chellappan, S., et al. 1991. The E2F transcription factor is a cellular target for the Rb protein. *Cell* 65: 1053-1061.
3. 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.
4. Bandara, L., et al. 1991. Cyclin A and the retinoblastoma gene product complex with a common transcription factor. *Nature* 352: 249-251.
5. Helin, K., et al. 1992. A cDNA encoding a pRb-binding protein with properties of the transcription factor E2F. *Cell* 70: 337-350.

CHROMOSOMAL LOCATION

Genetic locus: Rb12 (mouse) mapping to 8 C5.

PRODUCT

p130 siRNA (m) 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 p130 shRNA Plasmid (m): sc-29426-SH and p130 shRNA (m) Lentiviral Particles: sc-29426-V as alternate gene silencing products.

For independent verification of p130 (m) gene silencing results, we also provide the individual siRNA duplex components. Each is available as 3.3 nmol of lyophilized siRNA. These include: sc-29426A, sc-29426B and sc-29426C.

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

p130 siRNA (m) is recommended for the inhibition of p130 expression in mouse 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

p130 (A-10): sc-374521 is recommended as a control antibody for monitoring of p130 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 p130 gene expression knockdown using RT-PCR Primer: p130 (m)-PR: sc-29426-PR (20 μ l, 429 bp). Annealing temperature for the primers should be 55-60° C and the extension temperature should be 68-72° C.

SELECT PRODUCT CITATIONS

1. Zeng, L., et al. 2006. HDAC3 is crucial in shear- and VEGF-induced stem cell differentiation toward endothelial cells. *J. Cell Biol.* 174: 1059-1069.
2. Alessio, N., et al. 2017. misidentified human gene functions with mouse models: the case of the retinoblastoma gene family in senescence. *Neoplasia* 19: 781-790.

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.