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Rbx1 shRNA (h) Lentiviral Particles: sc-44072-V

BACKGROUND

Rbx1 (also designated ROC1 and Hrt1) and the closely related protein Rbx2 (also designated ROC2) are RING finger containing homologs of the yeast protein APC11, a member of the anaphase-promoting complex (APC). Rbx1 was shown to be a component of the von Hippel-Lindau (VHL) transcription elongation complex, which includes VHL, Elongin B, Elongin C and Cullin-2. Rbx1 interacts with Cullin-1 in the SCF (Skp1-Cdc53-F-box protein) ubiquitin ligase complex. Rbx1 functions as a common subunit of SCF complexes required for ubiquitination of various proteins including yeast G₁ cyclins, IκBα and β-catenin. Rbx1 was shown to enhance the ubiquitin ligase activity of the VHL/Cullin-2 complex, and of the SCF/Cullin-1 complex.

REFERENCES

1. Kamura, T., et al. 1999. Rbx1, a component of the VHL tumor suppressor complex and SCF ubiquitin ligase. *Science* 284: 657-661.
2. Tan, P., et al. 1999. Recruitment of a ROC1-CUL1 ubiquitin ligase by Skp1 and HOS to catalyze the ubiquitination of IκBα. *Mol. Cell* 3: 527-533.
3. Ohta, T., et al. 1999. ROC1, a homolog of APC11, represents a family of cullin partners with an associated ubiquitin ligase activity. *Mol. Cell* 3: 535-541.
4. Seol, J.H., et al. 1999. Cdc53/cullin and the essential Hrt1 RING-H2 subunit of SCF define a ubiquitin ligase module that activates the E2 enzyme Cdc34. *Genes Dev.* 13: 1614-1626.
5. Iwai, K., et al. 1999. Identification of the von hippel-lindau tumor-suppressor protein as part of an active E3 ubiquitin ligase complex. *Proc. Natl. Acad. Sci. USA* 96: 12436-12441.

CHROMOSOMAL LOCATION

Genetic locus: RBX1 (human) mapping to 22q13.2.

PRODUCT

Rbx1 shRNA (h) Lentiviral Particles is a pool of concentrated, transduction-ready viral particles containing 3 target-specific constructs that encode 19-25 nt (plus hairpin) shRNA designed to knock down gene expression. Each vial contains 200 μl frozen stock containing 1.0 x 10⁶ infectious units of virus (IFU) in Dulbecco's Modified Eagle's Medium with 25 mM HEPES pH 7.3. Suitable for 10-20 transductions. Also see Rbx1 siRNA (h): sc-44072 and Rbx1 shRNA Plasmid (h): sc-44072-SH as alternate gene silencing products.

STORAGE

Store lentiviral particles at -80° C. Stable for at least one year from the date of shipment. Once thawed, particles can be stored at 4° C for up to one week. Avoid repeated freeze thaw cycles.

PROTOCOLS

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

APPLICATIONS

Rbx1 shRNA (h) Lentiviral Particles is recommended for the inhibition of Rbx1 expression in human cells.

SUPPORT REAGENTS

Control shRNA Lentiviral Particles: sc-108080. Available as 200 μl frozen viral stock containing 1.0 x 10⁶ infectious units of virus (IFU); contains an shRNA construct encoding a scrambled sequence that will not lead to the specific degradation of any known cellular mRNA.

GENE EXPRESSION MONITORING

Rbx1 (E-11): sc-393640 is recommended as a control antibody for monitoring of Rbx1 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 (secondary) reagents are recommended: 1) Western Blotting: use goat anti-mouse IgG-HRP: sc-2005 (dilution range: 1:2000-1:32,000) or Cruz Marker™ compatible goat anti-mouse IgG-HRP: sc-2031 (dilution range: 1:2000-1:5000), Cruz Marker™ Molecular Weight Standards: sc-2035, TBS Blotto A Blocking Reagent: sc-2333 and Western Blotting Luminol Reagent: sc-2048. 2) Immunofluorescence: use goat anti-mouse IgG-FITC: sc-2010 (dilution range: 1:100-1:400) or goat anti-mouse IgG-TR: sc-2781 (dilution range: 1:100-1:400) with UltraCruz™ Mounting Medium: sc-24941.

RT-PCR REAGENTS

Semi-quantitative RT-PCR may be performed to monitor Rbx1 gene expression knockdown using RT-PCR Primer: Rbx1 (h)-PR: sc-44072-PR (20 μl). Annealing temperature for the primers should be 55-60° C and the extension temperature should be 68-72° C.

BIOSAFETY

Lentiviral particles can be employed in standard Biosafety Level 2 tissue culture facilities (and should be treated with the same level of caution as with any other potentially infectious reagent). Lentiviral particles are replication-incompetent and are designed to self-inactivate after transduction and integration of shRNA constructs into genomic DNA of target cells.

RESEARCH USE

The purchase of this product conveys to the buyer the nontransferable right to use the purchased amount of the product and all replicates and derivatives for research purposes conducted by the buyer in his laboratory only (whether the buyer is an academic or for-profit entity). The buyer cannot sell or otherwise transfer (a) this product (b) its components or (c) materials made using this product or its components to a third party, or otherwise use this product or its components or materials made using this product or its components for Commercial Purposes.