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Rbx1 siRNA (h): sc-44072

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 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 μM solution once resuspended using protocol below. Suitable for 50-100 transfections. Also see Rbx1 shRNA Plasmid (h): sc-44072-SH and Rbx1 shRNA (h) Lentiviral Particles: sc-44072-V as alternate gene silencing products.

For independent verification of Rbx1 (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-44072A, sc-44072B and sc-44072C.

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 μl of the RNase-free water provided. Resuspension of the siRNA duplex in 330 μl of RNase-free water makes a 10 μM solution in a 10 μM Tris-HCl, pH 8.0, 20 mM NaCl, 1 mM EDTA buffered solution.

APPLICATIONS

Rbx1 siRNA (h) is recommended for the inhibition of Rbx1 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 μM in 66 μl. 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

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 reagents are recommended: 1) Western Blotting: use m-IgGκ BP-HRP: sc-516102 or m-IgGκ 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-IgGκ BP-FITC: sc-516140 or m-IgGκ 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 Rbx1 gene expression knockdown using RT-PCR Primer: Rbx1 (h)-PR: sc-44072-PR (20 μl, 573 bp). Annealing temperature for the primers should be 55-60° C and the extension temperature should be 68-72° C.

SELECT PRODUCT CITATIONS

1. Marconett, C.N., et al. 2010. Indole-3-carbinol triggers aryl hydrocarbon receptor-dependent estrogen receptor (ER)α protein degradation in breast cancer cells disrupting an ERα-GATA3 transcriptional cross-regulatory loop. *Mol. Biol. Cell* 21: 1166-1177.

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.