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RNF181 siRNA (m): sc-153028

BACKGROUND

The RING-type zinc finger motif is present in a number of viral and eukaryotic proteins and is made of a conserved cysteine-rich domain that is able to bind two zinc atoms. Proteins that contain this conserved domain are generally involved in the ubiquitination pathway of protein degradation. RNF181 (RING finger protein 181) is a 153 amino acid protein that contains one RING-type zinc finger and belongs to the RNF181 family. RNF181 is auto-ubiquitinated as part of the enzymatic reaction, and upon DNA damage, RNF181 is phosphorylated by ATM or ATR. RNF181 demonstrates highest levels of expression in liver and heart, moderate levels in placenta, lung, liver, kidney, pancreas, and lower levels in brain and skeletal muscle. The RNF181 gene is conserved in chimpanzee, dog, cow, mouse, rat, zebrafish and fruit fly, and maps to human chromosome 2p11.2.

REFERENCES

1. Borden, K.L., et al. 1996. The RING finger domain: a recent example of a sequence-structure family. *Curr. Opin. Struct. Biol.* 6: 395-401.
2. Lorick, K.L., et al. 1999. RING fingers mediate ubiquitin-conjugating enzyme (E2)-dependent ubiquitination. *Proc. Natl. Acad. Sci. USA* 96: 11364-11369.
3. Zhang, Q.H., et al. 2000. Cloning and functional analysis of cDNAs with open reading frames for 300 previously undefined genes expressed in CD34⁺ hematopoietic stem/progenitor cells. *Genome Res.* 10: 1546-1560.
4. Strausberg, R.L., et al. 2002. Generation and initial analysis of more than 15,000 full-length human and mouse cDNA sequences. *Proc. Natl. Acad. Sci. USA* 99: 16899-16903.
5. Matsuoka, S., et al. 2007. ATM and ATR substrate analysis reveals extensive protein networks responsive to DNA damage. *Science* 316: 1160-1166.

CHROMOSOMAL LOCATION

Genetic locus: Rnf181 (mouse) mapping to 6 C1.

PRODUCT

RNF181 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 RNF181 shRNA Plasmid (m): sc-153028-SH and RNF181 shRNA (m) Lentiviral Particles: sc-153028-V as alternate gene silencing products.

For independent verification of RNF181 (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-153028A, sc-153028B and sc-153028C.

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.

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

RNF181 siRNA (m) is recommended for the inhibition of RNF181 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

RNF181 (F-2): sc-376343 is recommended as a control antibody for monitoring of RNF181 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 RNF181 gene expression knockdown using RT-PCR Primer: RNF181 (m)-PR: sc-153028-PR (20 μ l). Annealing temperature for the primers should be 55-60° C and the extension temperature should be 68-72° C.