

RNF5 siRNA (m): sc-106983

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. RNF5 (Ring finger protein 5), also known as RMA1, NG2, G16 or RING5, is a 180 amino acid protein that localizes to the membrane and contains one RING-type zinc finger. Expressed in a wide variety of tissues, RNF5 functions as a ubiquitin ligase that can regulate cell motility by controlling the ubiquitination of paxillin, a focal adhesion phosphoprotein that is localized to the cytoskeleton. RNF5 can target paxillin for ubiquitination, thereby altering the localization of paxillin and effecting its ability to recruit signaling molecules to focal adhesions. While overexpression of RNF5 is associated with the progression of breast cancer, lowered expression levels are observed in muscular disorders, indicating an important role for RNF5 in the regulation of cytoskeletal activity.

REFERENCES

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2. Kyushiki, H., et al. 1997. Cloning, expression and mapping of a novel RING-finger gene (RNF5), a human homologue of a putative zinc-finger gene from *Caenorhabditis elegans*. *Cytogenet. Cell Genet.* 79: 114-117.
3. Lorick, K.L., et al. 1999. RING fingers mediate ubiquitin-conjugating enzyme (E2)-dependent ubiquitination. *Proc. Natl. Acad. Sci. USA* 96: 11364-11369.
4. 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.
5. Matsuda, N., et al. 2001. Rma1, a novel type of RING finger protein conserved from *Arabidopsis* to human, is a membrane-bound ubiquitin ligase. *J. Cell Sci.* 114: 1949-1957.
6. Didier, C., et al. 2003. RNF5, a RING finger protein that regulates cell motility by targeting paxillin ubiquitination and altered localization. *Mol. Cell. Biol.* 23: 5331-5345.

CHROMOSOMAL LOCATION

Genetic locus: Rnf5 (mouse) mapping to 17 B1.

PRODUCT

RNF5 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 RNF5 shRNA Plasmid (m): sc-106983-SH and RNF5 shRNA (m) Lentiviral Particles: sc-106983-V as alternate gene silencing products.

For independent verification of RNF5 (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-106983A, sc-106983B and sc-106983C.

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

RNF5 siRNA (m) is recommended for the inhibition of RNF5 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

RNF5 (22B3): sc-81716 is recommended as a control antibody for monitoring of RNF5 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 RNF5 gene expression knockdown using RT-PCR Primer: RNF5 (m)-PR: sc-106983-PR (20 μ l). 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.