

RNF40 siRNA (h): sc-93054

BACKGROUND

Ubiquitination is an important mechanism through which three classes of enzymes act in concert to target short-lived or abnormal proteins for destruction. The three classes of enzymes involved in ubiquitination are the ubiquitin-activating enzymes (E1s), the ubiquitin-conjugating enzymes (E2s) and the ubiquitin-protein ligases (E3s). RNF40 (RING finger protein 40), also known as BRE1B, Staring or RBP95, is a 1,001 amino acid nuclear protein that contains one RING-type zinc finger. Expressed ubiquitously with highest expression in heart, testis and pancreas, RNF40 functions as an E3 ubiquitin-protein ligase that regulates the monoubiquitination and subsequent degradation of select residues on target proteins, such as Histone H2B and Syntaxin 1. In addition, RNF40 forms a ubiquitin ligase complex with UBC6 (an E2 enzyme) and together, these proteins play a crucial role in regulation of the histone code. Four isoforms of RNF40 exist due to alternative splicing events.

REFERENCES

1. Wen, H. and Ao, S. 2000. RBP95, a novel leucine zipper protein, binds to the retinoblastoma protein. *Biochem. Biophys. Res. Commun.* 275: 141-148.
2. Li, D., Day, K.V., Yu, S., Shi, G., Liu, S., Guo, M., Xu, Y., Sreedharan, S. and O'Malley, B.W. 2002. The role of adenovirus-mediated retinoblastoma 94 in the treatment of head and neck cancer. *Cancer Res.* 62: 4637-4644.
3. Chin, L.S., Vavalle, J.P. and Li, L. 2002. Staring, a novel E3 ubiquitin-protein ligase that targets Syntaxin 1 for degradation. *J. Biol. Chem.* 277: 35071-35079.
4. Online Mendelian Inheritance in Man, OMIM™. 2002. Johns Hopkins University, Baltimore, MD. MIM Number: 607700. World Wide Web URL: <http://www.ncbi.nlm.nih.gov/omim/>
5. Hwang, W.W., Venkatasubrahmanyam, S., Ianculescu, A.G., Tong, A., Boone, C. and Madhani, H.D. 2003. A conserved RING finger protein required for Histone H2B monoubiquitination and cell size control. *Mol. Cell* 11: 261-266.
6. Wood, A., Krogan, N.J., Dover, J., Schneider, J., Heidt, J., Boateng, M.A., Dean, K., Golshani, A., Zhang, Y., Greenblatt, J.F., Johnston, M. and Shilatifard, A. 2003. BRE1, an E3 ubiquitin ligase required for recruitment and substrate selection of Rad6 at a promoter. *Mol. Cell* 11: 267-274.

CHROMOSOMAL LOCATION

Genetic locus: RNF40 (human) mapping to 16p11.2.

PRODUCT

RNF40 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 μ M solution once resuspended using protocol below. Suitable for 50-100 transfections. Also see RNF40 shRNA Plasmid (h): sc-93054-SH and RNF40 shRNA (h) Lentiviral Particles: sc-93054-V as alternate gene silencing products.

For independent verification of RNF40 (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-93054A, sc-93054B and sc-93054C.

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

RNF40 siRNA (h) is recommended for the inhibition of RNF40 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 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

RNF40 (1C1): sc-293333 is recommended as a control antibody for monitoring of RNF40 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 RNF40 gene expression knockdown using RT-PCR Primer: RNF40 (h)-PR: sc-93054-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.