

MARCH6 siRNA (h): sc-91789

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). MARCH6 (membrane-associated ring finger (C3HC4) 6), also known as RNF176 or TEB4, is a 910 amino acid multi-pass membrane protein that localizes to the endoplasmic reticulum and contains one RING-CH-type zinc finger. Expressed in brain tissue, MARCH6 functions as an E3 ubiquitin-protein ligase that accepts a ubiquitin residue from an E2 ubiquitin-conjugating enzyme and transfers that ubiquitin residue to target proteins. MARCH6 exists as multiple alternative spliced isoforms and is subject to auto-ubiquitination, an event which results in its proteasomal degradation.

REFERENCES

1. Ciechanover, A. 1994. The ubiquitin-proteasome proteolytic pathway. *Cell* 79: 13-21.
2. Ciechanover, A., et al. 1994. The ubiquitin-mediated proteolytic pathway: mechanisms of recognition of the proteolytic substrate and involvement in the degradation of native cellular proteins. *FASEB J.* 8: 182-191.
3. Hochstrasser, M. 1995. Ubiquitin, proteasomes and the regulation of intracellular protein degradation. *Curr. Opin. Cell Biol.* 7: 215-223.
4. Liakopoulos, D., et al. 1998. A novel protein modification pathway related to the ubiquitin system. *EMBO J.* 17: 2208-2214.

CHROMOSOMAL LOCATION

Genetic locus: MARCH6 (human) mapping to 5p15.2.

PRODUCT

MARCH6 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 MARCH6 shRNA Plasmid (h): sc-91789-SH and MARCH6 shRNA (h) Lentiviral Particles: sc-91789-V as alternate gene silencing products.

For independent verification of MARCH6 (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-91789A, sc-91789B and sc-91789C.

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

MARCH6 siRNA (h) is recommended for the inhibition of MARCH6 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

MARCH6 (1A5): sc-517051 is recommended as a control antibody for monitoring of MARCH6 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 MARCH6 gene expression knockdown using RT-PCR Primer: MARCH6 (h)-PR: sc-91789-PR (20 μ l). Annealing temperature for the primers should be 55-60° C and the extension temperature should be 68-72° C.

SELECT PRODUCT CITATIONS

1. Nguyen, K.T., et al. 2019. N-terminal acetylation and the N-end rule pathway control degradation of the lipid droplet protein PLIN2. *J. Biol. Chem.* 294: 379-388.

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.