SANTA CRUZ BIOTECHNOLOGY, INC.

RFFL siRNA (m): sc-152823

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). RFFL, also known as RNF189, RNF34L or Rififylin, is a 363 amino acid protein that localizes to both the cytoplasm and to the membrane and contains one FYVE-type zinc finger, one RING-type zinc finger and 2 SAP domains. Expressed ubiquitously with increased expression in testis, spleen, ovary and prostate, RFFL functions as an E3 ubiquitin-protein ligase that interacts with caspase-8 and caspase-10 and targets them for proteasomal degradation. Multiple isoforms of RFFL exist due to alternative splicing events.

REFERENCES

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2. Coumailleau, F., et al. 2004. Over-expression of Rififylin, a new RING finger and FYVE-like domain-containing protein, inhibits recycling from the endocytic recycling compartment. Mol. Biol. Cell 15: 4444-4456.
3. McDonald, E.R. and El-Deiry, W.S. 2004. Suppression of caspase-8- and -10-associated RING proteins results in sensitization to death ligands and inhibition of tumor cell growth. Proc. Natl. Acad. Sci. USA 101: 6170-6175.
4. Beausoleil, S.A., et al. 2004. Large-scale characterization of HeLa cell nuclear phosphoproteins. Proc. Natl. Acad. Sci. USA 101: 12130-12135.
5. Tibbetts, M.D., et al. 2004. Crystal structure of a FYVE-type zinc finger domain from the caspase regulator CARP-2. Structure 12: 2257-2263.
6. Coumailleau, F., et al. 2005. Inhibition of endocytic recycling by Rififylin. Med. Sci. 21: 235-237.
7. Liao, W., et al. 2008. CARP-2 is an endosome-associated ubiquitin ligase for RIP and regulates TNF-induced NFκB activation. Curr. Biol. 18: 641-649.

CHROMOSOMAL LOCATION

Genetic locus: Rffl (mouse) mapping to 11 C.

PRODUCT

RFFL 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 µM solution once resuspended using protocol below. Suitable for 50-100 transfections. Also see RFFL shRNA Plasmid (m): sc-152823-SH and RFFL shRNA (m) Lentiviral Particles: sc-152823-V as alternate gene silencing products.

For independent verification of RFFL (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-152823A, sc-152823B and sc-152823C.

RESEARCH USE

For research use only, not for use in diagnostic procedures.

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

RFFL siRNA (m) is recommended for the inhibition of RFFL 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 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.

RT-PCR REAGENTS

Semi-quantitative RT-PCR may be performed to monitor RFFL gene expression knockdown using RT-PCR Primer: RFFL (m)-PR: sc-152823-PR (20 µl). Annealing temperature for the primers should be 55-60° C and the extension temperature should be 68-72° C.

PROTOCOLS

See our web site at www.scbt.com for detailed protocols and support products.