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Ubr4 siRNA (m): sc-154875

BACKGROUND

Ubr4 (ubiquitin protein ligase E3 component N-recognin 4), also known as p600, ZUBR1 or RBAF600 is a 5,183 amino acid multi-pass membrane protein that is concentrated at the edge of membrane structures that participate in Actin motility. Involved in protein degradation events, Ubr4 functions as an E3 ubiquitin-protein ligase that recognizes and binds proteins that contain specific N-terminal residues, thereby tagging the target proteins for ubiquitination and subsequent degradation. Additionally, Ubr4 interacts with Clathrin and forms meshwork structures that are used during cytoskeletal organization and morphogenesis. Ubr4 contains one Ubr-type zinc finger and is also thought to play a role in integrin-mediated signaling pathways. Six isoforms of Ubr4 exist due to alternative splicing events.

REFERENCES

1. Online Mendelian Inheritance in Man, OMIM[™]. 2002. Johns Hopkins University, Baltimore, MD. MIM Number: 609890. World Wide Web URL: <http://www.ncbi.nlm.nih.gov/omim/>
2. Tasaki, T., et al. 2005. A family of mammalian E3 ubiquitin ligases that contain the Ubr box motif and recognize N-degrons. *Mol. Cell. Biol.* 25: 7120-7136.
3. Huh, K.W., et al. 2005. Association of the human papillomavirus type 16 E7 oncoprotein with the 600 kDa retinoblastoma protein-associated factor, p600. *Proc. Natl. Acad. Sci. USA* 102: 11492-11497.
4. Nakatani, Y., et al. 2005. p600, a unique protein required for membrane morphogenesis and cell survival. *Proc. Natl. Acad. Sci. USA* 102: 15093-15098.
5. Tasaki, T., et al. 2007. Biochemical and genetic studies of Ubr3, a ubiquitin ligase with a function in olfactory and other sensory systems. *J. Biol. Chem.* 282: 18510-18520.
6. Lee, M.J., et al. 2008. Synthetic heterovalent inhibitors targeting recognition E3 components of the N-end rule pathway. *Proc. Natl. Acad. Sci. USA* 105: 100-105.

CHROMOSOMAL LOCATION

Genetic locus: Ubr4 (mouse) mapping to 4 D3.

PRODUCT

Ubr4 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 Ubr4 shRNA Plasmid (m): sc-154875-SH and Ubr4 shRNA (m) Lentiviral Particles: sc-154875-V as alternate gene silencing products.

For independent verification of Ubr4 (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-154875A, sc-154875B and sc-154875C.

PROTOCOLS

See our web site at www.scbt.com or our catalog 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

Ubr4 siRNA (m) is recommended for the inhibition of Ubr4 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

Ubr4 (E-14): sc-162376 is recommended as a control antibody for monitoring of Ubr4 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 (secondary) reagents are recommended: 1) Western Blotting: use donkey anti-goat IgG-HRP: sc-2020 (dilution range: 1:2000-1:100,000) or Cruz Marker[™] compatible donkey anti-goat IgG-HRP: sc-2033 (dilution range: 1:2000-1:5000), Cruz Marker[™] Molecular Weight Standards: sc-2035, TBS Blotto A Blocking Reagent: sc-2333 and Western Blotting Luminol Reagent: sc-2048. 2) Immunofluorescence: use donkey anti-goat IgG-FITC: sc-2024 (dilution range: 1:100-1:400) or donkey anti-goat IgG-TR: sc-2783 (dilution range: 1:100-1:400) with UltraCruz[™] Mounting Medium: sc-24941.

RT-PCR REAGENTS

Semi-quantitative RT-PCR may be performed to monitor Ubr4 gene expression knockdown using RT-PCR Primer: Ubr4 (m)-PR: sc-154875-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.