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SANTA CRUZ BIOTECHNOLOGY, INC.

Ubr3 siRNA (m): sc-155573

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). Ubr3 (Ubiquitin-protein ligase E3-α-3), also known as N-recognin-3 and Zinc finger protein 650, is a 1,888 amino acid multi-pass membrane protein that contains one UBR-type zinc finger and one RING-type zinc finger. Participating in protein modification events within the N-end rule pathway, Ubr1 and Ubr2 function as E3 ubiquitin-protein ligase that recognize and bind proteins that contain destabilizing N-terminal residues, thereby leading to their ubiquitination and subsequent degradation. Unlike its family members, Ubr3 does not recognize N-end rule substrates, but is rather thought to recognize small compounds that modulate the targeting of its subtrates. Adult mice that lack Ubr3 exhibit female-specific anosmia, suggesting that Ubr3 plays a regulatory role in sensory pathways like olfaction. There are four isoforms of Ubr3 that are produced as a result of alternative slicing events.

REFERENCES

1. Varshavsky, A. 1997. The N-end rule pathway of protein degradation. Genes Cells 2: 13-28.
2. Kwon, Y.T., et al. 1998. The mouse and human genes encoding the recognition component of the N-end rule pathway. Proc. Natl. Acad. Sci. USA 95: 7898-7903.
3. Ardley, H.C. and Robinson, P.A. 2005. E3 ubiquitin ligases. Essays Biochem. 41: 15-30.
4. 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.
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. Tasaki, T. and Kwon, Y.T. 2007. The mammalian N-end rule pathway: new insights into its components and physiological roles. Trends Biochem. Sci. 32: 520-528.

CHROMOSOMAL LOCATION

Genetic locus: Ubr3 (mouse) mapping to 2 C2.

PRODUCT

Ubr3 siRNA (m) is a pool of 2 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 Ubr3 shRNA Plasmid (m): sc-155573-SH and Ubr3 shRNA (m) Lentiviral Particles: sc-155573-V as alternate gene silencing products.

For independent verification of Ubr3 (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-155573A and sc-155573B.

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

Ubr3 siRNA (m) is recommended for the inhibition of Ubr3 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 60 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.

GENE EXPRESSION MONITORING

Ubr3 (5A10): sc-517094 is recommended as a control antibody for monitoring of Ubr3 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-IgGκ BP-HRP: sc-516102 or m-IgGκ 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-IgGκ BP-FITC: sc-516140 or m-IgGκ 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 Ubr3 gene expression knockdown using RT-PCR Primer: Ubr3 (m)-PR: sc-155573-PR (20 µl). 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.