

Ubr2 siRNA (h): sc-95444

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). Ubr2 (Ubiquitin-protein ligase E3- α -2), also known as N-recognin-2, is a 1,755 amino acid 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, Ubr2 functions as an E3 ubiquitin-protein ligase that recognizes and binds proteins that contain destabilizing N-terminal residues, thereby leading to their ubiquitination and subsequent degradation. Mice lacking Ubr2 are infertile due to defects in male meiosis.

REFERENCES

1. 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.
2. Ouyang, Y., et al. 2006. Loss of Ubr2, an E3 ubiquitin ligase, leads to chromosome fragility and impaired homologous recombinational repair. *Mutat. Res.* 596: 64-75.
3. An, J.Y., et al. 2006. Impaired neurogenesis and cardiovascular development in mice lacking the E3 ubiquitin ligases UBR1 and UBR2 of the N-end rule pathway. *Proc. Natl. Acad. Sci. USA* 103: 6212-6217.
4. 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.
5. Tasaki, T., et al. 2009. The substrate recognition domains of the N-end rule pathway. *J. Biol. Chem.* 284: 1884-1895.
6. Kume, K., et al. 2010. Role of N-end rule ubiquitin ligases UBR1 and UBR2 in regulating the leucine-mTOR signaling pathway. *Genes Cells* 15: 339-349.
7. An, J.Y., et al. 2010. UBR2 mediates transcriptional silencing during spermatogenesis via histone ubiquitination. *Proc. Natl. Acad. Sci. USA* 107: 1912-1917.

CHROMOSOMAL LOCATION

Genetic locus: UBR2 (human) mapping to 6p21.1.

PRODUCT

Ubr2 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 Ubr2 shRNA Plasmid (h): sc-95444-SH and Ubr2 shRNA (h) Lentiviral Particles: sc-95444-V as alternate gene silencing products.

For independent verification of Ubr2 (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-95444A, sc-95444B and sc-95444C.

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

Ubr2 siRNA (h) is recommended for the inhibition of Ubr2 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

Ubr2 (8H10): sc-135594 is recommended as a control antibody for monitoring of Ubr2 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 Ubr2 gene expression knockdown using RT-PCR Primer: Ubr2 (h)-PR: sc-95444-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.