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Ubr1 siRNA (m): sc-41688

BACKGROUND

The N-end rule relates the *in vivo* half-life of a protein to the composition of its N-terminal residues. The N-end rule pathway is part of the ubiquitin system, which involves a three-step mechanism. Proteins targeted for degradation are bound on their N-terminal residue by Ubr1 (also designated E3 α and N-recogin), which catalyzes the covalent attachment of ubiquitin to the protein substrate. Two zinc finger domains and the RING-H2 finger domain of Ubr1 are essential for substrate recognition. Ubr1 is located on mouse chromosome 2 E5 and on human chromosome 15q15.2 in the syntenic region. Ubr1 is ubiquitously expressed in adult mouse, with the highest expression detected in skeletal muscle and heart. In mouse embryo, Ubr1 is primarily expressed in the branchial arches and in the tail and limb buds.

REFERENCES

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- 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.
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CHROMOSOMAL LOCATION

Genetic locus: Ubr1 (mouse) mapping to 2 E5.

PRODUCT

Ubr1 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 Ubr1 shRNA Plasmid (m): sc-41688-SH and Ubr1 shRNA (m) Lentiviral Particles: sc-41688-V as alternate gene silencing products.

For independent verification of Ubr1 (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-41688A, sc-41688B and sc-41688C.

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.

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

Ubr1 siRNA (m) is recommended for the inhibition of Ubr1 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

Ubr1 (A-5): sc-515753 is recommended as a control antibody for monitoring of Ubr1 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 Ubr1 gene expression knockdown using RT-PCR Primer: Ubr1 (m)-PR: sc-41688-PR (20 μ l, 555 bp). Annealing temperature for the primers should be 55-60° C and the extension temperature should be 68-72° C.