

Ubiquitin siRNA (m): sc-36770

BACKGROUND

Ubiquitin (Ub) is among the most phylogenetically conserved proteins known. The primary function of Ubiquitin is to clear abnormal, foreign and improperly folded proteins by targeting them for degradation by the 26S Proteasome. This small, 76 amino acid protein can be covalently attached to cellular proteins via an isopeptide linkage between the carboxy terminal group of Ubiquitin and lysine amino groups on the acceptor protein. For proteolysis to occur, Ubiquitin oligomers must be assembled. Ubiquitin chains on proteolytic substrates are commonly found to have an isopeptide bridge between Lys 48 of one Ubiquitin molecule and the carboxy-terminus of a neighboring Ubiquitin molecule. Ubiquitin also plays a role in regulating signal transduction cascades through the elimination inhibitory proteins, such as I κ B- α and p27.

REFERENCES

1. Ciechanover, A. 1994. The Ubiquitin-proteasome proteolytic pathway. *Cell* 79: 13-21.
2. Ciechanover, A. and Schwartz, A.L. 1994. The Ubiquitin-mediated proteolytic pathway: mechanisms of recognition of the proteolytic substrate and involvement in the degradation of native cellular proteins. *FASEB J.* 8: 182-191.
3. Hochstrasser, M. 1995. Ubiquitin, proteasomes and the regulation of intracellular protein degradation. *Curr. Opin. Cell Biol.* 7: 215-223.
4. Pagano, M., et al. 1995. Role of the Ubiquitin-proteasome pathway in regulating abundance of the cyclin-dependent kinase inhibitor p27. *Science* 269: 682-685.

CHROMOSOMAL LOCATION

Genetic locus: Rps27a (mouse) mapping to 11 A3.3.

PRODUCT

Ubiquitin siRNA (m) is a target-specific 19-25 nt siRNA 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 Ubiquitin shRNA Plasmid (m): sc-36770-SH and Ubiquitin shRNA (m) Lentiviral Particles: sc-36770-V as alternate gene silencing 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

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

Ubiquitin (P4D1): sc-8017 is recommended as a control antibody for monitoring of Ubiquitin 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 MarkerTM 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 Ubiquitin gene expression knockdown using RT-PCR Primer: Ubiquitin (m)-PR: sc-36770-PR (20 μ l, 440 bp). Annealing temperature for the primers should be 55-60° C and the extension temperature should be 68-72° C.

SELECT PRODUCT CITATIONS

1. Frank, B., et al. 2015. Autophagic digestion of *Leishmania major* by host macrophages is associated with differential expression of BNIP3, CTSE, and the miRNAs miR-101c, miR-129, and miR-210. *Parasit. Vectors* 8: 404.
2. Ye, Y., et al. 2015. Inhibition of p-I κ B α ubiquitylation by autophagy-related gene 7 to regulate inflammatory responses to bacterial infection. *J. Infect. Dis.* 212: 1816-1826.
3. Hu, C.F., et al. 2021. K48-linked ubiquitination contributes to nicotine-augmented bone marrow-derived dendritic-cell-mediated adaptive immunity. *Vaccines* 9: 278.
4. Jia, J.J., et al. 2022. K48- and K27-mutant ubiquitin regulates adaptive immune response by affecting cross-presentation in bone marrow precursor cells. *J. Leukoc. Biol.* 112: 157-172.

RESEARCH USE

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