

Derlin-1 siRNA (m): sc-60520

BACKGROUND

Degradation in endoplasmic reticulum proteins, also designated derlins or DERtrins, are crucial for the degradation of misfolded endoplasmic reticulum (ER) luminal proteins. Derlin proteins are multipass membrane proteins localizing to the ER. Derlins are involved in transferring misfolded proteins from the ER to the cytosol, where the misfolded proteins are destroyed in a ubiquitin-dependent manner by the proteasome. In the case of cytomegalovirus infection, Derlin-1, as opposed to Derlins-2 and -3, is involved in the export of MHC class I heavy chains from the ER via its interaction with the viral protein US11. Derlins may also be important for cell proliferation. They are widely expressed, but highest levels are primarily detected in spleen, pancreas, lung, liver, thymus and ovary. Derlin-2 is overexpressed in hepatocarcinomas.

REFERENCES

1. Ying, H., et al. 2001. Cloning and characterization of F-LANA, upregulated in human liver cancer. *Biochem. Biophys. Res. Commun.* 286: 394-400.
2. Lilley, B.N. and Ploegh, H.L. 2004. A membrane protein required for dislocation of misfolded proteins from the ER. *Nature* 429: 834-840.
3. Ye, Y., et al. 2004. A membrane protein complex mediates retrotranslocation from the ER lumen into the cytosol. *Nature* 429: 841-847.
4. Katiyar, S., et al. 2005. The retrotranslocation protein Derlin-1 binds peptide: N-glycanase to the endoplasmic reticulum. *Mol. Biol. Cell* 16: 4584-4594.
5. Lilley, B.N. and Ploegh, H.L. 2005. Multiprotein complexes that link dislocation, ubiquitination and extraction of misfolded proteins from the endoplasmic reticulum membrane. *Proc. Natl. Acad. Sci. USA* 102: 14296-14301.
6. Oda, Y., et al. 2006. Derlin-2 and Derlin-3 are regulated by the mammalian unfolded protein response and are required for ER-associated degradation. *J. Cell Biol.* 172: 383-393.
7. Loureiro, J., et al. 2006. Signal peptide peptidase is required for dislocation from the endoplasmic reticulum. *Nature* 441: 894-897.
8. SWISS-PROT/TrEMBL (Q9GZP9). World Wide Web URL: <http://www.expasy.ch/sprot/sprot-top.html>

CHROMOSOMAL LOCATION

Genetic locus: Derl1 (mouse) mapping to 15 D1.

PRODUCT

Derlin-1 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 Derlin-1 shRNA Plasmid (m): sc-60520-SH and Derlin-1 shRNA (m) Lentiviral Particles: sc-60520-V as alternate gene silencing products.

For independent verification of Derlin-1 (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-60520A, sc-60520B and sc-60520C.

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

Derlin-1 siRNA (m) is recommended for the inhibition of Derlin-1 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

Derlin-1 (1B9): sc-293385 is recommended as a control antibody for monitoring of Derlin-1 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 Derlin-1 gene expression knockdown using RT-PCR Primer: Derlin-1 (m)-PR: sc-60520-PR (20 μ l, 568 bp). 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.