

Synoviolin siRNA (m): sc-76621

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). Synoviolin, also known as SYVN1 (synovial apoptosis inhibitor 1) or HRD1, is a 617 amino acid multi-pass membrane protein that localizes to the endoplasmic reticulum and contains one RING-type zinc finger. Expressed ubiquitously with highest expression in kidney and liver, Synoviolin exists as a homodimer that exhibits E3 ubiquitin-protein ligase activity and is a component of the ER-associated degradation (ERAD) complex, a multi-protein structure that mediates the degradation of misfolded proteins within the ER. Synoviolin is upregulated in patients with rheumatoid arthritis (RA), suggesting a role for Synoviolin in the pathogenesis of RA.

REFERENCES

1. Kikkert, M., et al. 2004. Human HRD1 is an E3 ubiquitin ligase involved in degradation of proteins from the endoplasmic reticulum. *J. Biol. Chem.* 279: 3525-3534.
2. 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.
3. Yamasaki, S., et al. 2006. Resistance to endoplasmic reticulum stress is an acquired cellular characteristic of rheumatoid synovial cells. *Int. J. Mol. Med.* 18: 113-117.
4. Yamasaki, S., et al. 2007. The roles of Synoviolin in crosstalk between endoplasmic reticulum stress-induced apoptosis and p53 pathway. *Cell Cycle* 6: 1319-1323.
5. Hosokawa, N., et al. 2008. Human XTP3-B forms an endoplasmic reticulum quality control scaffold with the HRD1-SEL1L ubiquitin ligase complex and BiP. *J. Biol. Chem.* 283: 20914-20924.

CHROMOSOMAL LOCATION

Genetic locus: Synv1 (mouse) mapping to 19 A.

PRODUCT

Synoviolin 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 Synoviolin shRNA Plasmid (m): sc-76621-SH and Synoviolin shRNA (m) Lentiviral Particles: sc-76621-V as alternate gene silencing products.

For independent verification of Synoviolin (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-76621A, sc-76621B and sc-76621C.

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

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

Synoviolin (4H4): sc-293484 is recommended as a control antibody for monitoring of Synoviolin 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 Synoviolin gene expression knockdown using RT-PCR Primer: Synoviolin (m)-PR: sc-76621-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.