

HtrA2 siRNA (m): sc-35616

BACKGROUND

The human homolog of the *E. Coli* HtrA gene product HtrA is identified in osteoarthritic cartilage and is repressed in SV40-transformed fibroblast. The gene encoding HtrA protein is highly conserved among mammalian species and belongs to the Serine protease family. The HtrA protein contains an IGF-binding domain and exhibits endoproteolytic activity, including autocatalytic cleavage. HtrA is a secreted protein that is expressed in heterologous systems. HtrA plays a role in the degradation of denatured proteins and cell growth regulation. Human HtrA2 (also designated Omi) is a novel member of the HtrA Serine protease family and is highly homologous to HtrA (also known as L56 and HtrA1). HtrA2 is ubiquitously expressed nuclear protease that is capable of autoproteolysis. The HtrA2 protein exists as two polypeptides and as an alternatively spliced form called D-Omi, which is predominately expressed in the kidney, colon and thyroid. Due to a modified PDZ domain, D-Omi does not interact with the known partner of HtrA2, the Mxi2 protein. Like HtrA, HtrA2 is involved in the degradation aberrantly folded proteins during conditions of cellular stress, suggesting that it may possess a chaperone-like role under normal conditions.

REFERENCES

1. Zumbrunn, J. and Trueb, B. 1996. Primary structure of a putative Serine protease specific for IGF-binding proteins. *FEBS Lett.* 398: 187-192.
2. Hu, S.I., et al. 1998. Human HtrA, an evolutionarily conserved Serine protease identified as a differentially expressed gene product in osteoarthritic cartilage. *J. Biol. Chem.* 273: 34406-34412.
3. Gray, C.W., et al. 2000. Characterization of human HtrA2, a novel Serine protease involved in the mammalian cellular stress response. *Eur. J. Biochem.* 267: 5699-5710.
4. Faccio, L., et al. 2000. Tissue-specific splicing of Omi stress-regulated endoprotease leads to an inactive protease with a modified PDZ motif. *Genomics* 68: 343-347.
5. Savopoulos, J.W., et al. 2000. Expression, purification, and functional analysis of the human Serine protease HtrA2. *Protein Expr. Purif.* 19: 227-234.

CHROMOSOMAL LOCATION

Genetic locus: Htra2 (mouse) mapping to 6 C3.

PRODUCT

HtrA2 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 HtrA2 shRNA Plasmid (m): sc-35616-SH and HtrA2 shRNA (m) Lentiviral Particles: sc-35616-V as alternate gene silencing products.

For independent verification of HtrA2 (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-35616A, sc-35616B and sc-35616C.

RESEARCH USE

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

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

HtrA2 siRNA (m) is recommended for the inhibition of HtrA2 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

HtrA2 (C-10): sc-271528 is recommended as a control antibody for monitoring of HtrA2 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 HtrA2 gene expression knockdown using RT-PCR Primer: HtrA2 (m)-PR: sc-35616-PR (20 μ l, 585 bp). Annealing temperature for the primers should be 55-60° C and the extension temperature should be 68-72° C.

PROTOCOLS

See our web site at www.scbt.com for detailed protocols and support products.