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HtrA shRNA (h) Lentiviral Particles: sc-43854-V

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 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 a 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 of aberrantly folded proteins during conditions of cellular stress, suggesting that it may possess a chaperone-like role under normal conditions.

REFERENCES

- Zumbrunn, J., et al. 1996. Primary structure of a putative serine protease specific for IGF-binding proteins. *FEBS Lett.* 398: 187-192.
- 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.
- 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.
- 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.
- 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: HTRA1 (human) mapping to 10q26.13.

PRODUCT

HtrA shRNA (h) Lentiviral Particles is a pool of concentrated, transduction-ready viral particles containing 3 target-specific constructs that encode 19-25 nt (plus hairpin) shRNA designed to knock down gene expression. Each vial contains 200 μ l frozen stock containing 1.0×10^6 infectious units of virus (IFU) in Dulbecco's Modified Eagle's Medium with 25 mM HEPES pH 7.3. Suitable for 10-20 transductions. Also see HtrA siRNA (h): sc-43854 and HtrA shRNA Plasmid (h): sc-43854-SH as alternate gene silencing products.

STORAGE

Store lentiviral particles at -80° C. Stable for at least one year from the date of shipment. Once thawed, particles can be stored at 4° C for up to one week. Avoid repeated freeze thaw cycles.

APPLICATIONS

HtrA shRNA (h) Lentiviral Particles is recommended for the inhibition of HtrA expression in human cells.

SUPPORT REAGENTS

Control shRNA Lentiviral Particles: sc-108080. Available as 200 μ l frozen viral stock containing 1.0×10^6 infectious units of virus (IFU); contains an shRNA construct encoding a scrambled sequence that will not lead to the specific degradation of any known cellular mRNA.

GENE EXPRESSION MONITORING

HtrA (B-8): sc-377050 is recommended as a control antibody for monitoring of HtrA 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 (secondary) reagents are recommended: 1) Western Blotting: use goat anti-mouse IgG-HRP: sc-2005 (dilution range: 1:2000-1:32,000) or Cruz Marker™ compatible goat anti-mouse IgG-HRP: sc-2031 (dilution range: 1:2000-1:5000), Cruz Marker™ Molecular Weight Standards: sc-2035, TBS Blotto A Blocking Reagent: sc-2333 and Western Blotting Luminol Reagent: sc-2048. 2) Immunofluorescence: use goat anti-mouse IgG-FITC: sc-2010 (dilution range: 1:100-1:400) or goat anti-mouse IgG-TR: sc-2781 (dilution range: 1:100-1:400) with UltraCruz™ Mounting Medium: sc-24941.

RT-PCR REAGENTS

Semi-quantitative RT-PCR may be performed to monitor HtrA gene expression knockdown using RT-PCR Primer: HtrA (h)-PR: sc-43854-PR (20 μ l). Annealing temperature for the primers should be $55-60^{\circ}$ C and the extension temperature should be $68-72^{\circ}$ C.

BIOSAFETY

Lentiviral particles can be employed in standard Biosafety Level 2 tissue culture facilities (and should be treated with the same level of caution as with any other potentially infectious reagent). Lentiviral particles are replication-incompetent and are designed to self-inactivate after transduction and integration of shRNA constructs into genomic DNA of target cells.

RESEARCH USE

The purchase of this product conveys to the buyer the nontransferable right to use the purchased amount of the product and all replicates and derivatives for research purposes conducted by the buyer in his laboratory only (whether the buyer is an academic or for-profit entity). The buyer cannot sell or otherwise transfer (a) this product (b) its components or (c) materials made using this product or its components to a third party, or otherwise use this product or its components or materials made using this product or its components for Commercial Purposes.

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