SANTA CRUZ BIOTECHNOLOGY, INC.

ClpP siRNA (m): sc-60414

BACKGROUND

ATP-dependent proteases were first identified in *E. coli*. One of these is called ClpAP or Ti, which consists of a regulatory unit, ClpA, with chaperone characteristics and an ATPase domain, and a proteolytic subunit, ClpP. This protease is involved in ATP-dependent degradation of incorrectly folded or unfolded proteins. The mammalian ClpP protein belongs to the peptidase family S14 and hydrolyzes proteins into small peptides in the presence of ATP and magnesium. ClpP is transported into mitochondrial matrix and is associated with the inner mitochondrial membrane. The functional form of ClpP is a hollowcored particle composed of two heptameric rings joined face-to-face forming an aqueous chamber containing the proteolytic active sites. ClpX binds substrates bearing specific classes of peptide signals, denatures these proteins, and translocates them through the central pore of ClpP for degradation. ClpP displays high expression levels in skeletal muscle, intermediate levels in heart, liver, and pancreas, and low levels in brain, placenta, lung, and kidney.

REFERENCES

1. Bross, P., et al. 1996. Human ClpP protease: cDNA sequence, tissue-specific expression and chromosomal assignment of the gene. FEBS Lett. 377: 249-252.
2. Corydon, T.J., et al. 1998. A human homologue of *Escherichia coli* ClpP caseinolytic protease: recombinant expression, intracellular processing and subcellular localization. Biochem. J. 331: 309-316.
3. de Sagarra, M.R., et al. 1999. Mitochondrial localization and oligomeric structure of HClpP, the human homologue of *E. coli* ClpP. J. Mol. Biol. 292: 819-825.
4. Zhao, Q., et al. 2002. A mitochondrial specific stress response in mammalian cells. EMBO J. 21: 4411-4419.
5. Kang, S.G., et al. 2002. Functional proteolytic complexes of the human mitochondrial ATP-dependent protease, hClpXP. J. Biol. Chem. 277: 21095-21102.
6. Kang, S.G., et al. 2004. Crystallography and mutagenesis point to an essential role for the N-terminus of human mitochondrial ClpP. J. Struct. Biol. 148: 338-352.

CHROMOSOMAL LOCATION

Genetic locus: Clpp (mouse) mapping to 17 D.

PRODUCT

ClpP 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 µM solution once resuspended using protocol below. Suitable for 50-100 transfections. Also see ClpP shRNA Plasmid (m): sc-60414-SH and ClpP shRNA (m) Lentiviral Particles: sc-60414-V as alternate gene silencing products.

For independent verification of ClpP (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-60414A, sc-60414B and sc-60414C.

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 µl of the RNAse-free water provided. Resuspension of the siRNA duplex in 330 µl of RNAse-free water makes a 10 µM solution in a 10 µM Tris-HCl, pH 8.0, 20 mM NaCl, 1 mM EDTA buffered solution.

APPLICATIONS

ClpP siRNA (m) is recommended for the inhibition of ClpP 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 µM in 66 µl. 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

ClpP (B-12): sc-271284 is recommended as a control antibody for monitoring of ClpP 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-IgGκ BP-HRP: sc-516102 or m-IgGκ 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-IgGκ BP-FITC: sc-516140 or m-IgGκ 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 ClpP gene expression knockdown using RT-PCR Primer: ClpP (m)-PR: sc-60414-PR (20 µl). 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.