SANTA CRUZ BIOTECHNOLOGY, INC.

Legumain siRNA (m): sc-60931

BACKGROUND

Legumain, also known as LGMN, AEP (asparaginyl endopeptidase) or PRSC1, is a 433 amino acid protein that localizes to the lysosome and belongs to the peptidase C13 family. Expressed ubiquitously with particularly high expression in placenta, heart and kidney, Legumain functions as a cysteine protease that specifically catalyzes the hydrolysis of asparaginyl and aspartyl bonds. Additionally, Legumain is thought to be involved in the processing of bacterial proteins for MHC class II antigen presentation in the lysosomal/endosomal system. Legumain exists as both a precursor and a fully mature, active enzyme that is produced in dendritic cells. Overexpression of Legumain may be associated with the formation of solid tumors, suggesting a role for Legumain in carcinogenesis. Multiple isoforms of Legumain exist due to alternative splicing events.

REFERENCES

1. Tanaka, T., et al. 1996. Molecular cloning of a human cDNA encoding putative cysteine protease (PRSC1) and its chromosome assignment to 14q32.1. Cytogenet. Cell Genet. 74: 120-123.
2. Chen, J.M., et al. 1997. Cloning, isolation, and characterization of mammalian Legumain, an asparaginyl endopeptidase. J. Biol. Chem. 272: 8090-8098.
3. Online Mendelian Inheritance in Man, OMIM™. 2002. Johns Hopkins University, Baltimore, MD. MIM Number: 602620. World Wide Web URL: http://www.ncbi.nlm.nih.gov/omim/
4. Li, D.N., et al. 2003. Multistep autoactivation of asparaginyl endopeptidase *in vitro* and *in vivo*. J. Biol. Chem. 278: 38980-38990.
5. Burster, T., et al. 2004. Cathepsin G, and not the asparagine-specific endoprotease, controls the processing of myelin basic protein in lysosomes from human B lymphocytes. J. Immunol. 172: 5495-5503.
6. Murthy, R.V., et al. 2005. Legumain expression in relation to clinicopathologic and biological variables in colorectal cancer. Clin. Cancer Res. 11: 2293-2299.
7. Liu, Z., et al. 2008. Neuroprotective actions of PIKE-L by inhibition of SET proteolytic degradation by asparagine endopeptidase. Mol. Cell 29: 665-678.

CHROMOSOMAL LOCATION

Genetic locus: Lgmn (mouse) mapping to 12 E.

PRODUCT

Legumain 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 Legumain shRNA Plasmid (m): sc-60931-SH and Legumain shRNA (m) Lentiviral Particles: sc-60931-V as alternate gene silencing products.

For independent verification of Legumain (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-60931A, sc-60931B and sc-60931C.

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

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

Legumain (B-8): sc-133234 is recommended as a control antibody for monitoring of Legumain 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 Legumain gene expression knockdown using RT-PCR Primer: Legumain (m)-PR: sc-60931-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.