SANTA CRUZ BIOTECHNOLOGY, INC.

Calpain 8 siRNA (h): sc-78999

BACKGROUND

Calpains are a family of cytosolic calcium-regulated cysteine proteases that functions to regulate a wide variety of cellular processes. Calpain 8, also known as nCL-2, is mostly produced at the surface of gastric epithelia and duodenal goblet cells and is likely involved with trafficking across the membranes of cells on the gastric surface. Calpain 8 acts to proteolyze the β subunit of the cytosolic coat protein. Coat proteins (COPs) are six subunit complexes found on the surface of vesicles within a cell. Calpain 8 proteolysis of the β subunit releases the COP from the cell's Golgi body.

REFERENCES

1. Stenbeck, G., et al. 1993. β'-COP, a novel subunit of coatomer. EMBO J. 12: 2841-2845.
2. Lee, H.J., et al. 1998. Molecular cloning and characterization of a novel tissue-specific calpain predominantly expressed in the digestive tract. Biol. Chem. 379: 175-183.
3. Braun, C., et al. 1999. CAPN 8: isolation of a new mouse calpain-isoenzyme. Biochem. Biophys. Res. Commun. 260: 671-675.
4. Hata, S., et al. 2001. Both the conserved and the unique gene structure of stomach-specific calpains reveal processes of calpain gene evolution. J. Mol. Evol. 53: 191-203.
5. Hata, S., et al. 2006. Stomach-specific calpain, nCL-2, localizes in mucus cells and proteolyzes the β-subunit of coatomer complex, β-COP. J. Biol. Chem. 281: 11214-11224.

CHROMOSOMAL LOCATION

Genetic locus: CAPN8 (human) mapping to 1q41.

PRODUCT

Calpain 8 siRNA (h) 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 Calpain 8 shRNA Plasmid (h): sc-78999-SH and Calpain 8 shRNA (h) Lentiviral Particles: sc-78999-V as alternate gene silencing products.

For independent verification of Calpain 8 (h) gene silencing results, we also provide the individual siRNA duplex components. Each is available as 3.3 nmol of lyophilized siRNA. These include: sc-78999A, sc-78999B and sc-78999C.

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

Calpain 8 siRNA (h) is recommended for the inhibition of Calpain 8 expression in human 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

Calpain (B-8): sc-271856 is recommended as a control antibody for monitoring of Calpain 8 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 Calpain 8 gene expression knockdown using RT-PCR Primer: Calpain 8 (h)-PR: sc-78999-PR (20 µl). Annealing temperature for the primers should be 55-60° C and the extension temperature should be 68-72° C.

SELECT PRODUCT CITATIONS

1. Yu, D., et al. 2010. miR-451 protects against erythroid oxidant stress by repressing 14-3-3ζ. Genes Dev. 24: 1620-1633.

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.