SANTA CRUZ BIOTECHNOLOGY, INC.

Calpain 8 siRNA (m): sc-62666

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.
6. Hata, S., et al. 2007. Stomach-specific calpain, nCL-2/Calpain 8, is active without calpain regulatory subunit and oligomerizes through C2-like domains. J. Biol. Chem. 282: 27847-27856.

CHROMOSOMAL LOCATION

Genetic locus: Capn8 (mouse) mapping to 1 H5.

PRODUCT

Calpain 8 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 Calpain 8 shRNA Plasmid (m): sc-62666-SH and Calpain 8 shRNA (m) Lentiviral Particles: sc-62666-V as alternate gene silencing products.

For independent verification of Calpain 8 (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-62666A, sc-62666B and sc-62666C.

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.

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 (m) is recommended for the inhibition of Calpain 8 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

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 (m)-PR: sc-62666-PR (20 µl). Annealing temperature for the primers should be 55-60° C and the extension temperature should be 68-72° C.