SANTA CRUZ BIOTECHNOLOGY, INC.

Calpastatin siRNA (m): sc-29890

BACKGROUND

Calpains are nonlysosomal, calcium-activated intracellular cysteine proteases that mediate specific Ca^{2+}-dependent processes including cell fusion, mitosis and meiosis. Calpains are heterodimers of a small regulatory subunit and one of three large catalytic subunits, designated Calpain 1, Calpain 2 and Calpain p94. Calpain 1 is an intracellular calcium-dependent protease that cleaves cytoskeletal and submembranous proteins. Calpain-1 co-localizes with human leukocyte antigen-DR (HLA-DR) on activated microglia in the aging brain. Calpain influences the process of spermatogenesis and the events preceding fertilization, such as the acrosome reaction. Calpastatin regulates Calpain by inhibiting both the proteolytic activity of Calpain and its binding to membranes. Calpastatin exists in two types, tissue type and erythrocyte type, resulting from both alternative splicing and proteolytic processing.

REFERENCES

1. Murachi, T. 1984. Calcium-dependent proteinases and specific inhibitors: Calpain and Calpastatin. Biochem. Soc. Symp. 45: 149-167.
2. Takano, E., et al. 1991. Molecular diversity of erythrocyte Calpastatin. Biomed. Biochim. Acta 50: 517-521.
3. Takano, E., et al. 1993. Molecular diversity of Calpastatin in human erythroid cells. Arch. Biochem. Biophys. 303: 349-354.
4. Kawasaki, H., et al. 1996. Regulation of the Calpain-Calpastatin system by membranes (review). Mol. Membr. Biol. 13: 217-224.
5. Johnson, G.V., et al. 1997. Calpains: intact and active? Bioessays 19: 1011-1018.
6. Elce, J.S., et al. 1997. Autolysis, Ca^{2+} requirement, and heterodimer stability in m-Calpain. J. Biol. Chem. 272: 11268-11275.
7. Niapour, M., et al. 2008. Regulation of Calpain activity by c-Myc through Calpastatin and promotion of transformation in c-Myc-negative cells by Calpastatin suppression. J. Biol. Chem. 283: 21371-21381.

CHROMOSOMAL LOCATION

Genetic locus: Cast (mouse) mapping to 13 C1.

PRODUCT

Calpastatin 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 Calpastatin shRNA Plasmid (m): sc-29890-SH and Calpastatin shRNA (m) Lentiviral Particles: sc-29890-V as alternate gene silencing products.

For independent verification of Calpastatin (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-29890A, sc-29890B and sc-29890C.

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

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

Calpastatin (PI-11): sc-32324 is recommended as a control antibody for monitoring of Calpastatin 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 Calpastatin gene expression knockdown using RT-PCR Primer: Calpastatin (m)-PR: sc-29890-PR (20 µl, 460 bp). 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.