

Calpastatin siRNA (h): sc-29889

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.

CHROMOSOMAL LOCATION

Genetic locus: CAST (human) mapping to 5q15.

PRODUCT

Calpastatin 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 Calpastatin shRNA Plasmid (h): sc-29889-SH and Calpastatin shRNA (h) Lentiviral Particles: sc-29889-V as alternate gene silencing products.

For independent verification of Calpastatin (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-29889A, sc-29889B and sc-29889C.

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.

PROTOCOLS

See our web site at www.scbt.com for detailed protocols and support products.

APPLICATIONS

Calpastatin siRNA (h) is recommended for the inhibition of Calpastatin 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.

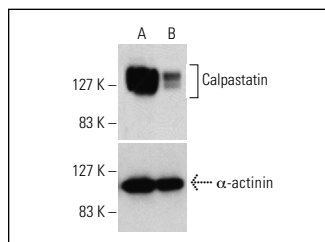
GENE EXPRESSION MONITORING

Calpastatin (A-1): sc-376547 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).

RT-PCR REAGENTS

Semi-quantitative RT-PCR may be performed to monitor Calpastatin gene expression knockdown using RT-PCR Primer: Calpastatin (h)-PR: sc-29889-PR (20 μl , 485 bp). Annealing temperature for the primers should be $55-60^{\circ}\text{C}$ and the extension temperature should be $68-72^{\circ}\text{C}$.

DATA



Calpastatin siRNA (h): sc-29889. Western blot analysis of Calpastatin expression in non-transfected control (A) and Calpastatin siRNA transfected (B) HeLa cells. Blot probed with Calpastatin (H-300): sc-20779. α -actinin (H-2): sc-17829 used as specificity and loading control.

SELECT PRODUCT CITATIONS

1. Li, C., et al. 2010. Proteasome inhibitor PS-341 (bortezomib) induces Calpain-dependent I κ B- α degradation. *J. Biol. Chem.* 285: 16096-16104.

RESEARCH USE

For research use only, not for use in diagnostic procedures.