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Calpain reg shRNA (m2) Lentiviral Particles: sc-44292-V

BACKGROUND

Calpain 1 is an intracellular calcium-dependent protease that cleaves cytoskeletal and submembranous proteins. Calpains are nonlysosomal, calcium-activated intracellular cysteine proteases. They 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. 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. 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.

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.
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4. Johnson, G.V., et al. 1997. Calpains: intact and active? *Bioessays* 19: 1011-1018.
5. Elce, J.S., et al. 1997. Autolysis, Ca^{2+} requirement, and heterodimer stability in m-calpain. *J. Biol. Chem.* 272: 11268-11275.
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CHROMOSOMAL LOCATION

Genetic locus: Capns1 (mouse) mapping to 7 B1.

PRODUCT

Calpain reg shRNA (m2) Lentiviral Particles is a pool of concentrated, transduction-ready viral particles containing 3 target-specific constructs that encode 19-25 nt (plus hairpin) shRNA designed to knock down gene expression. Each vial contains 200 μ l frozen stock containing 1.0×10^6 infectious units of virus (IFU) in Dulbecco's Modified Eagle's Medium with 25 mM HEPES pH 7.3. Suitable for 10-20 transductions. Also see Calpain reg siRNA (m2): sc-44292 and Calpain reg shRNA Plasmid (m2): sc-44292-SH as alternate gene silencing products.

STORAGE

Store lentiviral particles at -80° C. Stable for at least one year from the date of shipment. Once thawed, particles can be stored at 4° C for up to one week. Avoid repeated freeze thaw cycles.

PROTOCOLS

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

APPLICATIONS

Calpain reg shRNA (m2) Lentiviral Particles is recommended for the inhibition of Calpain reg expression in mouse cells.

SUPPORT REAGENTS

Control shRNA Lentiviral Particles: sc-108080. Available as 200 μ l frozen viral stock containing 1.0×10^6 infectious units of virus (IFU); contains an shRNA construct encoding a scrambled sequence that will not lead to the specific degradation of any known cellular mRNA.

GENE EXPRESSION MONITORING

Calpain reg (H-60): sc-30065 is recommended as a control antibody for monitoring of Calpain reg 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 (secondary) reagents are recommended: 1) Western Blotting: use goat anti-rabbit IgG-HRP: sc-2004 (dilution range: 1:2000-1:100,000) or Cruz Marker™ compatible goat anti-rabbit IgG-HRP: sc-2030 (dilution range: 1:2000-1:5000), Cruz Marker™ Molecular Weight Standards: sc-2035, TBS Blotto A Blocking Reagent: sc-2333 and Western Blotting Luminol Reagent: sc-2048. 2) Immunofluorescence: use goat anti-rabbit IgG-FITC: sc-2012 (dilution range: 1:100-1:400) or goat anti-rabbit IgG-TR: sc-2780 (dilution range: 1:100-1:400) with UltraCruz™ Mounting Medium: sc-24941.

RT-PCR REAGENTS

Semi-quantitative RT-PCR may be performed to monitor Calpain reg gene expression knockdown using RT-PCR Primer: Calpain reg (m2)-PR: sc-44292-PR (20 μ l). Annealing temperature for the primers should be $55-60^\circ$ C and the extension temperature should be $68-72^\circ$ C.

BIOSAFETY

Lentiviral particles can be employed in standard Biosafety Level 2 tissue culture facilities (and should be treated with the same level of caution as with any other potentially infectious reagent). Lentiviral particles are replication-incompetent and are designed to self-inactivate after transduction and integration of shRNA constructs into genomic DNA of target cells.

RESEARCH USE

The purchase of this product conveys to the buyer the nontransferable right to use the purchased amount of the product and all replicates and derivatives for research purposes conducted by the buyer in his laboratory only (whether the buyer is an academic or for-profit entity). The buyer cannot sell or otherwise transfer (a) this product (b) its components or (c) materials made using this product or its components to a third party, or otherwise use this product or its components or materials made using this product or its components for Commercial Purposes.