

Calpain 5 siRNA (m): sc-62065

BACKGROUND

Calpains are calcium-activated thiol proteases involved in intracellular processing of proteins and signal transduction. The classic calpains are heterodimers with one large subunit, one small subunit and five EF-hand-calcium binding structures. The large subunit varies between family members and can be active without the small subunit. Calpain 5, sometimes referred to as HTRA3, belongs to the non-classical calpain subfamily that lacks the EF-hand-calcium-binding structures. Calpain 5 is ubiquitously expressed, localizes to the cytoplasm and nucleus and is a homolog of Calpain 10. Calpain 5, in addition to Calpain 10 and Calpain 6, differs from other subfamily members by the presence of a T-domain that is homologous to the *Caenorhabditis elegans* protein Tra-3. Calpain 5, the human ortholog of this nematode protein, may play a role in sex determination.

REFERENCES

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3. Franz, T., et al. 2004. Capn5 is expressed in a subset of T cells and is dispensable for development. *Mol. Cell. Biol.* 24: 1649-1654.
4. Gafni, J., et al. 2004. Inhibition of calpain cleavage of Huntingtin reduces toxicity: accumulation of calpain/caspase fragments in the nucleus. *J. Biol. Chem.* 279: 20211-20220.
5. Waghray, A., et al. 2004. Molecular cloning and characterization of rat and human Calpain 5. *Biochem. Biophys. Res. Commun.* 324: 46-51.
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CHROMOSOMAL LOCATION

Genetic locus: Capn5 (mouse) mapping to 7 E2.

PRODUCT

Calpain 5 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 μ M solution once resuspended using protocol below. Suitable for 50-100 transfections. Also see Calpain 5 shRNA Plasmid (m): sc-62065-SH and Calpain 5 shRNA (m) Lentiviral Particles: sc-62065-V as alternate gene silencing products.

For independent verification of Calpain 5 (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-62065A, sc-62065B and sc-62065C.

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 μ l of the RNase-free water provided. Resuspension of the siRNA duplex in 330 μ l of RNase-free water makes a 10 μ M solution in a 10 μ M Tris-HCl, pH 8.0, 20 mM NaCl, 1 mM EDTA buffered solution.

APPLICATIONS

Calpain 5 siRNA (m) is recommended for the inhibition of Calpain 5 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 μ M in 66 μ l. 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 5 (A-5): sc-271271 is recommended as a control antibody for monitoring of Calpain 5 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-IgG κ BP-HRP: sc-516102 or m-IgG κ 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-IgG κ BP-FITC: sc-516140 or m-IgG κ 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 5 gene expression knockdown using RT-PCR Primer: Calpain 5 (m)-PR: sc-62065-PR (20 μ l). 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.

PROTOCOLS

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