

Calpain 3 siRNA (h): sc-41461

BACKGROUND

Calpain, an intracellular calcium-dependent protease that cleaves cytoskeletal and submembranous proteins, plays a role in cytoskeletal reorganization and muscle protein degradation. Calpain is a heterodimer composed of a small regulatory subunit and one of three large catalytic subunits, designated Calpain 1, Calpain 2 and Calpain 3. Calpain 3 (Calpain p94) is a muscle-preferred calcium activated neutral protease that localizes to the nucleus. The gene encoding human Calpain 3 maps to chromosome 15q15.1. Mutations involving the Calpain 3 gene are associated with limb-girdle muscle dystrophy type 2A, a form of autosomal recessive and progressive neuromuscular disorder. 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.
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4. Richard, I., et al. 1993. Mutations in the proteolytic enzyme Calpain 3 cause limb-girdle muscular dystrophy type 2A. *Cell* 81: 27-40.
5. Sorimachi, H., et al. 1993. Muscle-specific Calpain, p94, is degraded by autolysis immediately after translation, resulting in disappearance from muscle. *J. Biol. Chem.* 268: 10593-10605.
6. Johnson, G.V., et al. 1997. Calpains: intact and active? *Bioessays* 19: 1011-1018.
7. Elce, J.S., et al. 1997. Autolysis, Ca²⁺ requirement, and heterodimer stability in μ -Calpain. *J. Biol. Chem.* 272: 11268-11275.
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CHROMOSOMAL LOCATION

Genetic locus: CAPN3 (human) mapping to 15q15.1.

PRODUCT

Calpain 3 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 μ M solution once resuspended using protocol below. Suitable for 50-100 transfections. Also see Calpain 3 shRNA Plasmid (h): sc-41461-SH and Calpain 3 shRNA (h) Lentiviral Particles: sc-41461-V as alternate gene silencing products.

For independent verification of Calpain 3 (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-41461A, sc-41461B and sc-41461C.

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 3 siRNA (h) is recommended for the inhibition of Calpain 3 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 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 3 (E-6): sc-365277 is recommended as a control antibody for monitoring of Calpain 3 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 3 gene expression knockdown using RT-PCR Primer: Calpain 3 (h)-PR: sc-41461-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.