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- Trockeneiszuschlag
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# $\alpha$ -actinin-3 siRNA (h): sc-43099

## BACKGROUND

The spectrin gene family encodes a diverse group of cytoskeletal proteins that include spectrins, dystrophins and  $\alpha$ -actinins. There are four tissue-specific  $\alpha$ -actinins, namely  $\alpha$ -actinin-1,  $\alpha$ -actinin-2,  $\alpha$ -actinin-3 and  $\alpha$ -actinin-4, which are localized to muscle and non-muscle cells, including skeletal, cardiac and smooth muscle cells, as well as within the cytoskeleton. Each  $\alpha$ -actinin protein contains one Actin-binding domain, two calponin-homology domains, two EF-hand domains and four spectrin repeats, through which they function as bundling proteins that can cross-link F-Actin, thus anchoring Actin to a variety of intracellular structures. Defects in the gene encoding  $\alpha$ -actinin-4 are the cause of focal segmental glomerulosclerosis 1 (FSGS1), a common renal lesion characterized by decreasing kidney function and, ultimately, renal failure.

## REFERENCES

1. Yousoufian, H., et al. 1990. Cloning and chromosomal localization of the human cytoskeletal  $\alpha$ -actinin gene reveals linkage to the  $\beta$ -spectrin gene. *Am. J. Hum. Genet.* 47: 62-71.
2. Nishiyama, M., et al. 1990. Expression of human  $\alpha$ -actinin in human hepatocellular carcinoma. *Cancer Res.* 50: 6291-6294.
3. Yürüker, B. and Niggli, V. 1992.  $\alpha$ -actinin and vinculin in human neutrophils: reorganization during adhesion and relation to the Actin network. *J. Cell Sci.* 101: 403-414.
4. Knudsen, K.A., et al. 1995. Interaction of  $\alpha$ -actinin with the cadherin/catenin cell-cell adhesion complex via  $\alpha$ -catenin. *J. Cell Biol.* 130: 67-77.
5. Reinhard, M., et al. 1999. An  $\alpha$ -actinin binding site of Zyxin is essential for subcellular Zyxin localization and  $\alpha$ -actinin recruitment. *J. Biol. Chem.* 274: 13410-13418.
6. Harper, B.D., et al. 2000. Fine mapping of the  $\alpha$ -actinin binding site within cysteine-rich protein. *Biochem. J.* 350: 269-274.
7. Gonzalez, A.M., et al. 2001. Interactions of a hemidesmosome component and actinin family members. *J. Cell Sci.* 114: 4197-4206.
8. Bois, P.R., et al. 2005. Structural dynamics of  $\alpha$ -actinin-vinculin interactions. *Mol. Cell. Biol.* 25: 6112-6122.

## CHROMOSOMAL LOCATION

Genetic locus: ACTN3 (human) mapping to 11q13.2.

## PRODUCT

$\alpha$ -actinin-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  $\mu$ M solution once resuspended using protocol below. Suitable for 50-100 transfections. Also see  $\alpha$ -actinin-3 shRNA Plasmid (h): sc-43099-SH and  $\alpha$ -actinin-3 shRNA (h) Lentiviral Particles: sc-43099-V as alternate gene silencing products.

For independent verification of  $\alpha$ -actinin-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-43099A, sc-43099B and sc-43099C.

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

## APPLICATIONS

$\alpha$ -actinin-3 siRNA (h) is recommended for the inhibition of  $\alpha$ -actinin-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  $\mu$ M in 66  $\mu$ 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

$\alpha$ -actinin (H-2): sc-17829 is recommended as a control antibody for monitoring of  $\alpha$ -actinin-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 $\kappa$  BP-HRP: sc-516102 or m-IgG $\kappa$  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 $\kappa$  BP-FITC: sc-516140 or m-IgG $\kappa$  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  $\alpha$ -actinin-3 gene expression knockdown using RT-PCR Primer:  $\alpha$ -actinin-3 (h)-PR: sc-43099-PR (20  $\mu$ 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](http://www.scbt.com) for detailed protocols and support products.