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# nestin siRNA (r): sc-156055

## BACKGROUND

Nestin is a major intermediate filament (IF) protein of embryonic central nervous system progenitor cells. It is also a component of the dynamic IF network during muscle development, where it polymerizes with Desmin and Vimentin. Nestin co-assembles with Vimentin or  $\alpha$ -internexin and forms heterodimer coiled-coil molecules which then further assemble into 10 nm IFs. Deletion of the IF consensus rod domain in nestin alters nestin localization in CNS precursor cells and radial glial cells *in vivo*. Nestin is a marker for neuroepithelial stem cells, glioma cells and tumor endothelial cells during rapid growth. During axon elongation of differentiation neurons, nestin localizes to the growth cones and may play a role in growth cone guidance. In the rat adrenal gland, nestin is expressed by the zona fasciculata and the zona reticularis. Nestin is also expressed by dermal cells and by myoblasts during the earliest stages of myogenesis.

## REFERENCES

- Lendahl, U., et al. 1990. CNS stem cells express a new class of intermediate filament protein. *Cell* 60: 585-595.
- Sejersen, T., et al. 1993. Transient expression of the intermediate filament nestin during skeletal muscle development. *J. Cell Sci.* 106: 1291-1300.
- Kachinsky, A.M., et al. 1994. Myogenesis and the intermediate filament protein, nestin. *Dev. Biol.* 165: 216-228.
- Marvin, M.J., et al. 1998. A rod end deletion in the intermediate filament protein nestin alters its subcellular localization in neuroepithelial cells of transgenic mice. *J. Cell Sci.* 111: 1951-1961.
- Steinert, P.M., et al. 1999. A high molecular weight intermediate filament-associated protein in BHK-21 cells is nestin, a type VI intermediate filament protein. Limited co-assembly *in vitro* to form heteropolymers with type III vimentin and type IV  $\alpha$ -internexin. *J. Biol. Chem.* 274: 9881-9890.
- Yan, Y., et al. 2001. Mouse nestin protein localizes in growth cones of P19 neurons and cerebellar granule cells. *Neurosci. Lett.* 302: 89-92.
- Sugawara, K., et al. 2002. Nestin as a marker for proliferative endothelium in gliomas. *Lab. Invest.* 82: 345-351.
- Bertelli, E., et al. 2002. Nestin expression in rat adrenal gland. *Histochem. Cell Biol.* 117: 371-377.

## CHROMOSOMAL LOCATION

Genetic locus: Nes (rat) mapping to 2q34.

## PRODUCT

nestin siRNA (r) 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 nestin shRNA Plasmid (r): sc-156055-SH and nestin shRNA (r) Lentiviral Particles: sc-156055-V as alternate gene silencing products.

For independent verification of nestin (r) gene silencing results, we also provide the individual siRNA duplex components. Each is available as 3.3 nmol of lyophilized siRNA. These include: sc-156055A, sc-156055B and sc-156055C.

## 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

nestin siRNA (r) is recommended for the inhibition of nestin expression in rat 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

nestin (10c2): sc-23927 is recommended as a control antibody for monitoring of nestin 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<sup>™</sup> Molecular Weight Standards: sc-2035, UltraCruz<sup>®</sup> 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<sup>®</sup> Mounting Medium: sc-24941 or UltraCruz<sup>®</sup> Hard-set Mounting Medium: sc-359850.

## RT-PCR REAGENTS

Semi-quantitative RT-PCR may be performed to monitor nestin gene expression knockdown using RT-PCR Primer: nestin (r)-PR: sc-156055-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.