

# Axotrophin siRNA (h): sc-60235

## BACKGROUND

Axotrophin is a stem cell gene that encodes a protein which is involved in T lymphocyte regulation (especially in regulating the proliferation) and leukemia inhibitory factor (LIF) release. LIF is a neuropoietic cytokine that is important for stem cell regulation and thymocyte stimulation. Both Axotrophin and LIF are linked to transplantation intolerance. Axotrophin is also involved in corpus callosum differentiation and may play a role in glial cell line-derived neurotrophic factor (GDNF)-dependent sensory neuron survival in the substantia gelatinosa of the adult spinal cord. Axotrophin is primarily expressed in the hippocampus, cortex, purkinje and granule cells of the cerebellum.

## REFERENCES

1. Escary, J.L., et al. 1993. Leukaemia stem cells and thymocyte stimulation. *Nature* 363: 361-364.
2. Online Mendelian Inheritance in Man, OMIM<sup>™</sup>. 2002. Johns Hopkins University, Baltimore, MD. MIM Number: 159540. World Wide Web URL: <http://www.ncbi.nlm.nih.gov/omim/>
3. Metcalfe, S.M., et al. 2005. Transplantation tolerance: gene expression profiles comparing allotolerance vs. alloreactivity. *Int. Immunopharmacol.* 5: 33-39.
4. Metcalfe, S.M., et al. 2005. Leukaemia inhibitory factor (LIF) is functionally linked to Axotrophin and both LIF and Axotrophin are linked to regulatory immune tolerance. *FEBS Lett.* 579: 609-614.
5. Metcalfe, S.M. 2005. Axotrophin and leukaemia inhibitory factor (LIF) in transplantation tolerance. *Philos. Trans. R. Soc. Lond., B, Biol. Sci.* 360: 1687-1694.

## CHROMOSOMAL LOCATION

Genetic locus: MARCH7 (human) mapping to 2q24.2.

## PRODUCT

Axotrophin 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 Axotrophin shRNA Plasmid (h): sc-60235-SH and Axotrophin shRNA (h) Lentiviral Particles: sc-60235-V as alternate gene silencing products.

For independent verification of Axotrophin (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-60235A, sc-60235B and sc-60235C.

## 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

Axotrophin siRNA (h) is recommended for the inhibition of Axotrophin 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

Axotrophin (B-2): sc-166945 is recommended as a control antibody for monitoring of Axotrophin 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 Axotrophin gene expression knockdown using RT-PCR Primer: Axotrophin (h)-PR: sc-60235-PR (20  $\mu$ l, 612 bp). 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.