

# Agrin siRNA (r): sc-61875

## BACKGROUND

Agrin is a molecule that resides in the basal lamina of muscle cells and directs key events in post synaptic differentiation. Most notably, Agrin is responsible for the clustering of acetylcholine receptors (AChRs) on the cell surface and their localization to the neuromuscular junction. Several Agrin variants have been identified which arise from alternative mRNA splicings. Agrin splice forms having inserts at two sites in the carboxy terminus designated "y" and "z" display a high affinity for AChRs, while splice forms lacking these inserts associate with AChRs weakly. Muscle  $\alpha$ -dystroglycan has been postulated to be the receptor for the clustering activity of Agrin; however, this is a point of contention. Tyrosine phosphorylation has been implicated as a required early step in AChR aggregation. Interestingly, a unique receptor tyrosine kinase, designated MuSK, has been discovered that interacts with Agrin and is specifically localized to developing muscle.

## REFERENCES

1. Bowen, D.C., et al. 1996. Neural Agrin activates a high-affinity receptor in C2 muscle cells that is unresponsive to muscle Agrin. *J. Neurosci.* 16: 3791-3797.
2. Gautam, M., et al. 1996. Defective neuromuscular synaptogenesis in Agrin-deficient mutant mice. *Cell* 85: 525-535.
3. Slater, C.R. 1996. Agrin signals at the junction. *Nature* 381: 478-479.
4. Gesemann, M., et al. 1996. Alternative splicing of Agrin alters its binding to heparin, dystroglycan, and the putative Agrin receptor. *Neuron* 16: 755-767.
5. O'Toole, J.J., et al. 1996. Alternative splicing of Agrin regulates its binding to heparin  $\alpha$ -dystroglycan, and the cell surface. *Proc. Natl. Acad. Sci. USA* 93: 7369-7374.
6. Hopf, C. and Hoch, W. 1996. Agrin binding to  $\alpha$ -dystroglycan. Domains of Agrin necessary to induce acetylcholine receptor clustering are overlapping but not identical to the  $\alpha$ -dystroglycan-binding region. *J. Biol. Chem.* 271: 5231-5236.

## CHROMOSOMAL LOCATION

Genetic locus: Agrn (rat) mapping to 5q36.

## PRODUCT

Agrin 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 Agrin shRNA Plasmid (r): sc-61875-SH and Agrin shRNA (r) Lentiviral Particles: sc-61875-V as alternate gene silencing products.

For independent verification of Agrin (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-61875A, sc-61875B and sc-61875C.

## PROTOCOLS

See our web site at [www.scbt.com](http://www.scbt.com) for detailed protocols and support products.

## 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

Agrin siRNA (r) is recommended for the inhibition of Agrin 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

Agrin (D-2): sc-374117 is recommended as a control antibody for monitoring of Agrin 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 Agrin gene expression knockdown using RT-PCR Primer: Agrin (r)-PR: sc-61875-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.