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Ankyrin G siRNA (h): sc-43263

BACKGROUND

Members of the Ankyrin family of proteins mediate the attachment of integral membrane proteins to the cytoskeleton. ANK1, ANK2 and ANK3 genes encode for the proteins in this family, Ankyrin-1 (also designated Ankyrin R), Ankyrin B and Ankyrin G, respectively. The proteins are structured similarly, each composed of an N-terminal domain with multiple ankyrin repeats, a highly conserved central spectrin binding domain and C-terminal regulatory domains which are susceptible to the most variance. Both Ankyrin B and Ankyrin G are essential for normal neuronal functions. Ankyrin G was originally identified localized to the nodes of Ranvier and axon initial segments where it was found to mediate normal neuronal sodium channel function. Ankyrin G, named for its giant size and general expression, exhibits tissue-specific alternative mRNA processing. The two largest protein isoforms are expressed only in nervous tissue, with additional smaller isoforms found in an array of other tissues including lung, kidney, spleen, liver and heart.

REFERENCES

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2. Zhang, X., et al. 1998. Restriction of 480/270-kD Ankyrin G to axon proximal segments requires multiple Ankyrin G-specific domains. *J. Cell Biol.* 142: 1571-1581.
3. Bennett, V., et al. 1999. Physiological roles of axonal ankyrins in survival of premyelinated axons and localization of voltage-gated sodium channels. *J. Neurocytol.* 28: 303-318.
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6. Kretschmer, T., et al. 2002. Painful neuromas: a potential role for a structural transmembrane protein, Ankyrin G. *J. Neurosurg.* 97: 1424-1431.
7. Kizhatil, K., et al. 2004. Lateral membrane biogenesis in human bronchial epithelial cells requires 190-kDa Ankyrin G. *J. Biol. Chem.* 279: 16706-16714.

CHROMOSOMAL LOCATION

Genetic locus: ANK3 (human) mapping to 10q21.2.

PRODUCT

Ankyrin G 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 Ankyrin G shRNA Plasmid (h): sc-43263-SH and Ankyrin G shRNA (h) Lentiviral Particles: sc-43263-V as alternate gene silencing products.

For independent verification of Ankyrin G (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-43263A, sc-43263B and sc-43263C.

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

Ankyrin G siRNA (h) is recommended for the inhibition of Ankyrin G 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

Ankyrin G (463): sc-12719 is recommended as a control antibody for monitoring of Ankyrin G 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 Ankyrin G gene expression knockdown using RT-PCR Primer: Ankyrin G (h)-PR: sc-43263-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.