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Ankyrin G shRNA (h) Lentiviral Particles: sc-43263-V

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

1. Kordeli, E., Lambert, S. and Bennett, V. 1995. Ankyrin G. A new ankyrin gene with neural-specific isoforms localized at the axonal initial segment and node of Ranvier. *J. Biol. Chem.* 270: 2352-2359.
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.
4. Bongenhielm, U., et al. 2000. Expression of sodium channel SNS/PN3 and Ankyrin G mRNAs in the trigeminal ganglion after inferior alveolar nerve injury in the rat. *Exp. Neurol.* 164: 384-395.

CHROMOSOMAL LOCATION

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

PRODUCT

Ankyrin G shRNA (h) Lentiviral Particles is a pool of concentrated, transduction-ready viral particles containing 3 target-specific constructs that encode 19-25 nt (plus hairpin) shRNA designed to knock down gene expression. Each vial contains 200 µl frozen stock containing 1.0×10^6 infectious units of virus (IFU) in Dulbecco's Modified Eagle's Medium with 25 mM HEPES pH 7.3. Suitable for 10-20 transductions. Also see Ankyrin G siRNA (h): sc-43263 and Ankyrin G shRNA Plasmid (h): sc-43263-SH as alternate gene silencing products.

STORAGE

Store lentiviral particles at -80° C. Stable for at least one year from the date of shipment. Once thawed, particles can be stored at 4° C for up to one week. Avoid repeated freeze thaw cycles.

APPLICATIONS

Ankyrin G shRNA (h) Lentiviral Particles is recommended for the inhibition of Ankyrin G expression in human cells.

SUPPORT REAGENTS

Control shRNA Lentiviral Particles: sc-108080. Available as 200 µl frozen viral stock containing 1.0×10^6 infectious units of virus (IFU); contains an shRNA construct encoding a scrambled sequence that will not lead to the specific degradation of any known cellular mRNA.

GENE EXPRESSION MONITORING

Ankyrin G (H-4): sc-166602 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 (secondary) reagents are recommended: 1) Western Blotting: use goat anti-mouse IgG-HRP: sc-2005 (dilution range: 1:2000-1:32,000) or Cruz Marker™ compatible goat anti-mouse IgG-HRP: sc-2031 (dilution range: 1:2000-1:5000), Cruz Marker™ Molecular Weight Standards: sc-2035, TBS Blotto A Blocking Reagent: sc-2333 and Western Blotting Luminol Reagent: sc-2048. 2) Immunofluorescence: use goat anti-mouse IgG-FITC: sc-2010 (dilution range: 1:100-1:400) or goat anti-mouse IgG-TR: sc-2781 (dilution range: 1:100-1:400) with UltraCruz™ Mounting Medium: sc-24941.

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 µl). Annealing temperature for the primers should be 55-60° C and the extension temperature should be 68-72° C.

BIOSAFETY

Lentiviral particles can be employed in standard Biosafety Level 2 tissue culture facilities (and should be treated with the same level of caution as with any other potentially infectious reagent). Lentiviral particles are replication-incompetent and are designed to self-inactivate after transduction and integration of shRNA constructs into genomic DNA of target cells.

RESEARCH USE

The purchase of this product conveys to the buyer the nontransferable right to use the purchased amount of the product and all replicates and derivatives for research purposes conducted by the buyer in his laboratory only (whether the buyer is an academic or for-profit entity). The buyer cannot sell or otherwise transfer (a) this product (b) its components or (c) materials made using this product or its components to a third party, or otherwise use this product or its components or materials made using this product or its components for Commercial Purposes.