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Formin 2 shRNA (m) Lentiviral Particles: sc-45895-V

BACKGROUND

The temporal genetic hierarchy influencing normal limb development can deregulate and mediate mammalian developmental syndromes. In mice, the limb deformity (Id) locus influences normal limb development and gives rise to alternative mRNAs that can translate into a family of proteins known as formins. Formins play a crucial role in cytoskeletal reorganization by influencing Actin filament assembly. Formins co-localize with the actin cytoskeleton and can translocate into the cell cytosol and into the nucleus in an HGF-dependent manner. Vertebrate nuclear formins can control polarizing activity in limb buds through establishment of a Sonic hedgehog/FGF-4 feedback loop. Deficiency mutations at the mammalian Id locus lead to profound developmental defects in limb and kidney formation. The human Formin 1 and 2 genes map to chromosome 15q13.3 and 1q43, respectively.

REFERENCES

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2. Wynshaw-Boris, A., et al. 1997. The role of a single formin isoform in the limb and renal phenotypes of limb deformity. *Mol. Med.* 3: 372-384.
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4. Tanaka, K. 2000. Formin family proteins in cytoskeletal control. *Biochem. Biophys. Res. Commun.* 267: 479-481.
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CHROMOSOMAL LOCATION

Genetic locus: Fmn2 (mouse) mapping to 1 H3.

PRODUCT

Formin 2 shRNA (m) 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 μ l 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 Formin 2 siRNA (m): sc-45895 and Formin 2 shRNA Plasmid (m): sc-45895-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

Formin 2 shRNA (m) Lentiviral Particles is recommended for the inhibition of Formin 2 expression in mouse cells.

SUPPORT REAGENTS

Control shRNA Lentiviral Particles: sc-108080. Available as 200 μ l 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

Formin 2 (C-3): sc-376787 is recommended as a control antibody for monitoring of Formin 2 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 Formin 2 gene expression knockdown using RT-PCR Primer: Formin 2 (m)-PR: sc-45895-PR (20 μ l, 569 bp). Annealing temperature for the primers should be $55-60^\circ$ C and the extension temperature should be $68-72^\circ$ 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.

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