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Dynein HC shRNA (m) Lentiviral Particles: sc-44778-V

BACKGROUND

Dyneins are multisubunit, high molecular weight ATPases that interact with microtubules to generate force by converting the chemical energy of ATP into the mechanical energy of movement. Cytoplasmic or axonemal Dynein heavy, intermediate, light and light-intermediate chains are all components of minus end-directed motors; the complex transports cellular cargos towards the central region of the cell. Axonemal Dynein motors contain one to three non-identical heavy chains and cause a sliding of microtubules in the axonemes of cilia and flagella in a mechanism necessary for cilia to beat and propel the cell. Cytoplasmic Dynein is an approximately 12 subunit complex of two heavy chains, two intermediate chains to anchor Dynein to its cargo, four smaller intermediate chains and several light chains. It performs functions necessary for cell survival such as organelle transport and centrosome assembly. The carboxy terminus of Dynein is important for microtubule-dependent motility and is highly conserved, while the amino terminal regions are more variable. Several proteins regulate Dynein activity, including dynactin, LIS1 and NudEL (NudE-like).

REFERENCES

1. Mallik, R., et al. 2004. Cytoplasmic dynein functions as a gear in response to load. *Nature* 427: 649-652.
2. Malikov, V., et al. 2004. Cytoplasmic dynein nucleates microtubules to organize them into radial arrays *in vivo*. *Mol. Biol. Cell.* 15: 2742-2749.
3. Asai, D.J., et al. 2004. The dynein heavy chain family. *J. Eukaryot Microbiol.* 51: 23-29.
4. Li, J., et al. 2005. NudEL targets dynein to microtubule ends through LIS1. *Nat. Cell. Biol.* 7: 686-690.
5. Seetharam, R.N., et al. 2005. High speed sliding of axonemal microtubules produced by outer arm dynein. *Cell. Motil. Cytoskeleton.* 60: 96-103.
6. He, Y., et al. 2005. Role of cytoplasmic dynein in the axonal transport of microtubules and neurofilaments. *J. Cell Biol.* 168: 697-703.

CHROMOSOMAL LOCATION

Genetic locus: Dync1h1 (mouse) mapping to 12 F1.

PRODUCT

Dynein HC 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 Dynein HC siRNA (m): sc-44778 and Dynein HC shRNA Plasmid (m): sc-44778-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

Dynein HC shRNA (m) Lentiviral Particles is recommended for the inhibition of Dynein heavy chain 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

Dynein HC (R-325): sc-9115 is recommended as a control antibody for monitoring of Dynein heavy chain 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-rabbit IgG-HRP: sc-2004 (dilution range: 1:2000-1:100,000) or Cruz Marker™ compatible goat anti-rabbit IgG-HRP: sc-2030 (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-rabbit IgG-FITC: sc-2012 (dilution range: 1:100-1:400) or goat anti-rabbit IgG-TR: sc-2780 (dilution range: 1:100-1:400) with UltraCruz™ Mounting Medium: sc-24941.

RT-PCR REAGENTS

Semi-quantitative RT-PCR may be performed to monitor Dynein heavy chain gene expression knockdown using RT-PCR Primer: Dynein HC (m)-PR: sc-44778-PR (20 μ l, 568 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.

PROTOCOLS

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