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Dynein HC siRNA (h): sc-43738

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 2 heavy chains, 2 intermediate chains to anchor Dynein to its cargo, 4 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.

CHROMOSOMAL LOCATION

Genetic locus: DYNC1H1 (human) mapping to 14q32.31.

PRODUCT

Dynein HC 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 Dynein HC shRNA Plasmid (h): sc-43738-SH and Dynein HC shRNA (h) Lentiviral Particles: sc-43738-V as alternate gene silencing products.

For independent verification of Dynein HC (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-43738A, sc-43738B and sc-43738C.

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

Dynein HC siRNA (h) is recommended for the inhibition of Dynein HC 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

Dynein HC (C-5): sc-514579 is recommended as a control antibody for monitoring of Dynein HC 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 Dynein HC gene expression knockdown using RT-PCR Primer: Dynein HC (h)-PR: sc-43738-PR (20 μ l, 562 bp). Annealing temperature for the primers should be 55-60° C and the extension temperature should be 68-72° C.

SELECT PRODUCT CITATIONS

1. Nakano, H., et al. 2010. Nucleoporin translocated promoter region (Tpr) associates with Dynein complex, preventing chromosome lagging formation during mitosis. *J. Biol. Chem.* 285: 10841-10849.
2. Silva, P.M., et al. 2014. Dynein-dependent transport of spindle assembly checkpoint proteins off kinetochores toward spindle poles. *FEBS Lett.* 588: 3265-3273.

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.