SANTA CRUZ BIOTECHNOLOGY, INC.

Dynactin 5 siRNA (h): sc-93011

BACKGROUND

Dynactin is a multisubunit complex that functions as a binding partner for the Dynein microtubule motor. Dynactin-Dynein binding may be required for most, if not all, cytoplasmic Dynein-driven activities and is thought to contribute to the functional diversity of Dynein. Dynactin 5, also known as Dynactin p25, is an evolutionarily conserved component of the Arp1 filament pointed-end-binding subcomplex of the Dynactin shoulder complex. This pointed-end-binding subcomplex also consists of Dynactin 6, Dynactin p62 and ACTR10. Dynactin 5, along with Dynactin p62 and Dynactin 6, is believed to function in the regulation of Dynactin-membranous cargo interactions. Further supporting its role in cargo binding, Dynactin 5 is essential for retrograde vesicle trafficking. Dynactin 5 contains an isoleucine-patch motif and exhibits a left-handed parallel β-helix fold.

REFERENCES

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3. Parisi, G., Fornasari, M.S. and Echave, J. 2004. Dynactins p25 and p27 are predicted to adopt the LβH fold. FEBS Lett. 562: 1-4.
4. Hodgkinson, J.L., Peters, C., Kuznetsov, S.A. and Steffen, W. 2005. Three-dimensional reconstruction of the Dynactin complex by single-particle image analysis. Proc. Natl. Acad. Sci. USA 102: 3667-3672.
5. Levy, J.R. and Holzbaur, E.L. 2006. Cytoplasmic Dynein/Dynactin function and dysfunction in motor neurons. Int. J. Dev. Neurosci. 24: 103-111.
6. Hossain, Z., Ali, S.M., Ko, H.L., Xu, J., Ng, C.P., Guo, K., Qi, Z., Ponniah, S., Hong, W. and Hunziker, W. 2007. Glomerulocystic kidney disease in mice with a targeted inactivation of Wwtr1. Proc. Natl. Acad. Sci. USA 104: 1631-1636.

CHROMOSOMAL LOCATION

Genetic locus: DCTN5 (human) mapping to 16p12.2.

PRODUCT

Dynactin 5 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 µM solution once resuspended using protocol below. Suitable for 50-100 transfections. Also see Dynactin 5 shRNA Plasmid (h): sc-93011-SH and Dynactin 5 shRNA (h) Lentiviral Particles: sc-93011-V as alternate gene silencing products.

For independent verification of Dynactin 5 (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-93011A, sc-93011B and sc-93011C.

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 µl of the RNAse-free water provided. Resuspension of the siRNA duplex in 330 µl of RNAse-free water makes a 10 µM solution in a 10 µM Tris-HCl, pH 8.0, 20 mM NaCl, 1 mM EDTA buffered solution.

APPLICATIONS

Dynactin 5 siRNA (h) is recommended for the inhibition of Dynactin 5 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 µM in 66 µl. 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

Dynactin 5 (D-9): sc-518204 is recommended as a control antibody for monitoring of Dynactin 5 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-IgGκ BP-HRP: sc-516102 or m-IgGκ 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-IgGκ BP-FITC: sc-516140 or m-IgGκ 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 Dynactin 5 gene expression knockdown using RT-PCR Primer: Dynactin 5 (h)-PR: sc-93011-PR (20 µl). 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.