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Dynactin 1 siRNA (h): sc-43317

BACKGROUND

Dynactin, a multisubunit complex, is a cytoplasmic Dynein-interacting protein that functions as the "receptor" 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. Enriched in neurons, Dynactin also binds to microtubules and has been shown to function in diverse processes, including organelle transport, formation of the mitotic spindle and cytokinesis. Dynactin subunits include p22, p50, p62, p150 (also designated Glued) and ARP-1. The p135 splice variant is neuron specific and, unlike p150, does not bind microtubules.

REFERENCES

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- Tokito, M.K., et al. 1997. Functionally distinct isoforms of dynactin are expressed in human neurons. *Mol. Biol. Cell* 7: 1167-1180.
- Waterman-Storer, C.M., et al. 1997. The interaction between cytoplasmic dynein and dynactin is required for fast axonal transport. *Proc. Natl. Acad. Sci. USA* 94: 12180-12185.
- Holleran, E.A., et al. 1998. The role of the dynactin complex in intracellular motility. *Int. Rev. Cytol.* 182: 69-109.
- Karki, S., et al. 1998. Characterization of the p22 subunit of dynactin reveals the localization of cytoplasmic dynein and dynactin to the midbody of dividing cells. *J. Cell Biol.* 142: 1023-1034.
- Berrueta, L., et al. 1999. The APC-associated protein EB1 associates with components of the dynactin complex and cytoplasmic dynein intermediate chain. *Curr. Biol.* 9: 425-428.

CHROMOSOMAL LOCATION

Genetic locus: DCTN1 (human) mapping to 2p13.1.

PRODUCT

Dynactin 1 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 Dynactin 1 shRNA Plasmid (h): sc-43317-SH and Dynactin 1 shRNA (h) Lentiviral Particles: sc-43317-V as alternate gene silencing products.

For independent verification of Dynactin 1 (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-43317A, sc-43317B and sc-43317C.

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.

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

Dynactin 1 siRNA (h) is recommended for the inhibition of Dynactin 1 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

Dynactin 1 (A-2): sc-365274 is recommended as a control antibody for monitoring of Dynactin 1 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 Dynactin 1 gene expression knockdown using RT-PCR Primer: Dynactin 1 (h)-PR: sc-43317-PR (20 μ l, 595 bp). Annealing temperature for the primers should be 55-60° C and the extension temperature should be 68-72° C.