

Dynactin p62 siRNA (h): sc-35232

BACKGROUND

Dynactin is a multisubunit complex and a required cofactor for most, or all, of the cellular processes powered by the microtubule-based motor cytoplasmic dynein. Dynactin contains a short Actin-related protein 1 (Arp1) filament with capZ at the barbed end and p62 at the pointed end. The p62 subunit is an integral component of 20 S Dynactin with a highly conserved cysteine-rich motif that interacts directly with Arp1. Dynactin p62 has a punctate cytoplasmic distribution as well as centrosomal distribution typical of Dynactin. In addition, Dynactin p62 is distributed in the nucleus at very high expression levels. Due to the structural composition of Dynactin, the p62 subunit is implicated in Arp1 pointed-end binding and in linking Dynein and Dynactin to the cortical cytoskeleton.

REFERENCES

1. Schafer, D.A., et al. 1994. Ultrastructural analysis of the Dynactin complex: an Actin-related protein is a component of a filament that resembles F-Actin. *J. Cell Biol.* 126: 403-412.
2. Garces, J.A., et al. 1999. Interaction of the p62 subunit of Dynactin with Arp1 and the cortical Actin cytoskeleton. *Curr. Biol.* 9: 1497-1500.
3. Eckley, D.M., et al. 1999. Analysis of Dynactin subcomplexes reveals a novel Actin-related protein associated with the Arp1 minifilament pointed end. *J. Cell Biol.* 147: 307-320.
4. Bingham, J.B., et al. 1999. Self-regulated polymerization of the Actin-related protein Arp1. *Curr. Biol.* 9: 223-226.
5. Karki, S., et al. 2000. A Dynactin subunit with a highly conserved cysteine-rich motif interacts directly with Arp1. *J. Biol. Chem.* 275: 4834-4839.

CHROMOSOMAL LOCATION

Genetic locus: DCTN4 (human) mapping to 5q33.1.

PRODUCT

Dynactin p62 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 p62 shRNA Plasmid (h): sc-35232-SH and Dynactin p62 shRNA (h) Lentiviral Particles: sc-35232-V as alternate gene silencing products.

For independent verification of Dynactin p62 (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-35232A, sc-35232B and sc-35232C.

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 p62 siRNA (h) is recommended for the inhibition of Dynactin p62 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 p62 (H-4): sc-55603 is recommended as a control antibody for monitoring of Dynactin p62 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 p62 gene expression knockdown using RT-PCR Primer: Dynactin p62 (h)-PR: sc-35232-PR (20 μ l). Annealing temperature for the primers should be 55-60° C and the extension temperature should be 68-72° C.

SELECT PRODUCT CITATIONS

1. Zhang, Y., et al. 2017. Mcl-1 expression and JNK activation induces a threshold for apoptosis in Bcl-x_L-overexpressing hematopoietic cells. *Oncotarget* 8: 11042-11052.

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.