

Arp3 siRNA (m): sc-29740

BACKGROUND

Actin polymerization is required for a variety of cell functions, including chemotaxis, cell migration, cell adhesion, and platelet activation. Cells trigger Actin polymerization through either the *de novo* nucleation of filaments from monomeric Actin, the severing of existing filaments to create uncapped barbed ends, or the uncapping existing barbed ends. The nucleation of Actin is a rate-limiting and unfavorable reaction in Actin polymerization and therefore requires the involvement of the Arp2/3 complex, which helps create new filaments and promotes the end-to-side cross-linking of Actin filaments into the branching meshwork. The Arp2/3 complex consists of the Actin-related proteins Arp2 and Arp3, and various other accessory proteins. The Arp2/3 complex promotes Actin nucleation by binding the pointed end of Actin filaments, or by associating with the side of an existing filament, and nucleates growth in the barbed direction. In addition, the Arp2/3 complex also mediates Actin cytoskeletal outgrowths that are regulated by the Rho family of small GTPases. In response to GTP-binding Cdc42, the Arp2/3 complex binds the Cdc42 substrates, namely the WASP proteins, and initiates the formation of lamellipodia and filopodia.

REFERENCES

1. Mullins, R.D., et al. 1998. The interaction of Arp2/3 complex with actin: nucleation, high affinity pointed end capping, and formation of branching networks of filaments. *Proc. Natl. Acad. Sci. USA* 95: 6181-6186.
2. Bailly, M., et al. 1999. Relationship between Arp2/3 complex and the barbed ends of actin filaments at the leading edge of carcinoma cells after epidermal growth factor stimulation. *J. Cell Biol.* 145: 331-345.
3. Higgs, H.N., et al. 1999. Regulation of actin polymerization by Arp2/3 complex and WASp/Scar proteins. *J. Biol. Chem.* 274: 32531-32534.
4. Svitkina, T.M., et al. 1999. Arp2/3 complex and actin depolymerizing factor/cofilin in dendritic organization and treadmilling of actin filament array in lamellipodia. *J. Cell Biol.* 145: 1009-1026.
5. Egile, C., et al. 1999. Activation of the CDC42 effector N-WASP by the *Shigella flexneri* IcsA protein promotes actin nucleation by Arp2/3 complex and bacterial actin-based motility. *J. Cell Biol.* 146: 1319-1332.

CHROMOSOMAL LOCATION

Genetic locus: Actr3 (mouse) mapping to 1 E3.

PRODUCT

Arp3 siRNA (m) 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 Arp3 shRNA Plasmid (m): sc-29740-SH and Arp3 shRNA (m) Lentiviral Particles: sc-29740-V as alternate gene silencing products.

For independent verification of Arp3 (m) gene silencing results, we also provide the individual siRNA duplex components. Each is available as 3.3 nmol of lyophilized siRNA. These include: sc-29740A, sc-29740B and sc-29740C.

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

Arp3 siRNA (m) is recommended for the inhibition of Arp3 expression in mouse 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

Arp3 (A-1): sc-48344 is recommended as a control antibody for monitoring of Arp3 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 Arp3 gene expression knockdown using RT-PCR Primer: Arp3 (m)-PR: sc-29740-PR (20 μ l). 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.