



Cortactin shRNA Plasmid (h): sc-35093-SH

BACKGROUND

Cortactin (also designated Ems-1) is a filamentous Actin (F-Actin) binding protein that has been shown to be a substrate for Src p60. Cortactin contains tandem 37 amino acid repeats at the amino-terminus and an SH3 domain at the carboxy-terminus. The tandem repeats appear to be necessary for F-Actin binding. Tyrosine phosphorylation of Cortactin by Src p60 results in diminished F-Actin binding to Cortactin and reduced F-Actin cross-linking activity. Cortactin has also been shown to be phosphorylated in response to FGF-1. Cortactin exhibits abundant expression in megakaryocytes and platelets, and it may play a role in the maturation of megakaryocytes.

REFERENCES

1. Wu, H., et al. 1993. Cortactin, an 80/85-kilodalton pp60^{src} substrate, is a filamentous Actin-binding protein enriched in the cell cortex. *J. Cell Biol.* 120: 1417-1426.
2. Zhan, X., et al. 1993. Murine cortactin is phosphorylated in response to fibroblast growth factor-1 on tyrosine residues late in the G₁ phase of the BALB/c 3T3 cell cycle. *J. Biol. Chem.* 268: 24427-24431.
3. Zhan, X., et al. 1994. Association of fibroblast growth factor receptor-1 with c-Src correlates with association between c-Src and cortactin. *J. Biol. Chem.* 269: 20221-20224.
4. Okamura, H., et al. 1995. p80/85 Cortactin associates with the Src SH2 domain and colocalizes with v-Src in transformed cells. *J. Biol. Chem.* 270: 26613-26618.
5. Huang, C., et al. 1997. Down-regulation of the filamentous Actin cross-linking activity of cortactin by Src-mediated tyrosine phosphorylation. *J. Biol. Chem.* 272: 13911-13915.

CHROMOSOMAL LOCATION

Genetic locus: CTTN (human) mapping to 11q13.3.

PRODUCT

Cortactin shRNA Plasmid (h) is a pool of 3 target-specific lentiviral vector plasmids each encoding 19-25 nt (plus hairpin) shRNAs designed to knock down gene expression. Each plasmid contains a puromycin resistance gene for the selection of cells stably expressing shRNA. Each vial contains 20 µg of lyophilized shRNA plasmid DNA. Suitable for up to 20 transfections. Also see Cortactin siRNA (h): sc-35093 and Cortactin shRNA (h) Lentiviral Particles: sc-35093-V as alternate gene silencing products.

STORAGE AND RESUSPENSION

Store lyophilized shRNA plasmid DNA at 4° C with desiccant. Stable for at least one year from the date of shipment. Once resuspended, store at 4° C for short term storage or -80° C for long term storage. Avoid repeated freeze thaw cycles.

Resuspend lyophilized shRNA plasmid DNA in 200 µl of the deionized water provided. Resuspension of the shRNA plasmid DNA in 200 µl of deionized water makes a 0.1 µg/µl solution in a 10 mM Tris, 1 mM EDTA buffered solution.

APPLICATIONS

Cortactin shRNA Plasmid (h) is recommended for the inhibition of Cortactin expression in human cells.

SUPPORT REAGENTS

For optimal shRNA Plasmid transfection efficiency, Santa Cruz Biotechnology's shRNA Plasmid Transfection Reagent: sc-108061 (0.2 ml) and shRNA Plasmid Transfection Medium: sc-108062 (20 ml) are recommended. Control shRNAs are available as 20 µg lyophilized plasmid DNA. Each encodes a scrambled shRNA sequence that will not lead to the specific degradation of any known cellular mRNA. Control shRNA Plasmids include: sc-108060, sc-108065 and sc-108066.

GENE EXPRESSION MONITORING

Cortactin (H-5): sc-55579 is recommended as a control antibody for monitoring of Cortactin 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 Cortactin gene expression knockdown using RT-PCR Primer: Cortactin (h)-PR: sc-35093-PR (20 µl, 466 bp). Annealing temperature for the primers should be 55-60° C and the extension temperature should be 68-72° C.

RESEARCH USE

The purchase of this product conveys to the buyer the nontransferable right to use the purchased amount of the product and all replicates and derivatives for research purposes conducted by the buyer in his laboratory only (whether the buyer is an academic or for-profit entity). The buyer cannot sell or otherwise transfer (a) this product (b) its components or (c) materials made using this product or its components to a third party, or otherwise use this product or its components or materials made using this product or its components for Commercial Purposes.

PROTOCOLS

See our web site at www.scbt.com for detailed protocols and support products.