SANTA CRUZ BIOTECHNOLOGY, INC.

AFAP-110 siRNA (m): sc-40955

BACKGROUND

Actin filament associated protein (AFAP-110) interacts directly with Actin filaments through its C-terminal Actin binding domain. AFAP-110 contains additional protein-binding domains as well, and serves as an adaptor protein. AFAP-110 links signaling molecules to Actin filaments, provides a platform for the preparation of larger signaling complexes, activates Src kinases in response to cellular signals and also directly affects Actin organization as an Actin filament cross-linking protein. Deletion of certain binding elements of AFAP-110 results in altered Actin phenotypes; for instance, deletion of the leucine zipper motif causes repositioning of Actin into rosettes. Because inhibition of certain Actin cytoskeletal conformations inhibits cell division and movement, these conformational changes to AFAP-110, and thus Actin organization in the cell, represent a possible therapeutic target for controlling tumorigenesis and metastasis.

REFERENCES

1. Guappone, A.C., et al. 1997. The integrity of the SH3 binding motif of AFAP-110 is required to facilitate tyrosine phosphorylation by, and stable complex formation with, Src. Mol. Cell. Biochem. 175: 243-252.
2. Qian, Y., et al. 1998. Src can regulate carboxy terminal interactions with AFAP-110, which influence self-association, cell localization and Actin filament integrity. Oncogene 16: 2185-2195.
3. Guappone, A.C., et al. 1998. Formation of a stable Src-AFAP-110 complex through either an amino-terminal or a carboxy-terminal SH2-binding motif. Mol. Carcinog. 22: 110-119.
4. Qian, Y., et al. 2000. The carboxy terminus of AFAP-110 modulates direct interactions with Actin filaments and regulates its ability to alter Actin filament integrity and induce lamellipodia formation. Exp. Cell Res. 255: 102-113.
5. Baisden, J.M., et al. 2001. The Actin filament-associated protein AFAP-110 is an adaptor protein that modulates changes in Actin filament integrity. Oncogene 20: 6435-6447.
6. Baisden, J.M., et al. 2001. The intrinsic ability of AFAP-110 to alter Actin filament integrity is linked with its ability to also activate cellular tyrosine kinases. Oncogene 20: 6607-6616.

CHROMOSOMAL LOCATION

Genetic locus: Afap1 (mouse) mapping to 5 B3.

PRODUCT

AFAP-110 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 µM solution once resuspended using protocol below. Suitable for 50-100 transfections. Also see AFAP-110 shRNA Plasmid (m): sc-40955-SH and AFAP-110 shRNA (m) Lentiviral Particles: sc-40955-V as alternate gene silencing products.

For independent verification of AFAP-110 (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-40955A, sc-40955B and sc-40955C.

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

AFAP-110 siRNA (m) is recommended for the inhibition of AFAP-110 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 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

AFAP-110 (D-11): sc-374655 is recommended as a control antibody for monitoring of AFAP-110 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 AFAP-110 gene expression knockdown using RT-PCR Primer: AFAP-110 (m)-PR: sc-40955-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.