SANTA CRUZ BIOTECHNOLOGY, INC.

AFAP-1L1 siRNA (m): sc-140895

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. By linking signaling molecules to Actin filaments, AFAP-110 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. AFAP-1L1 (Actin filament-associated protein 1-like 1) is a 768 amino acid protein that, like its relative AFAP-110, contains two Pleckstrin homology (PH) domains, which are normally found in proteins involved in intracellular signaling. AFAP-1L1 is phosphorylated upon DNA damage, probably by ATR or Atm. There are four isoforms of AFAP-1L1 that are produced as a result of alternative splicing events.

REFERENCES

1. Musacchio, A., et al. 1993. The PH domain: a common piece in the structural patchwork of signalling proteins. Trends Biochem. Sci. 18: 343-348.
2. 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.
3. 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.
4. 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.
5. Lodyga, M., et al. 2002. Molecular cloning of actin filament-associated protein: a putative adaptor in stretch-induced Src activation. Am. J. Physiol. Lung Cell. Mol. Physiol. 283: L265-L274.
6. Jikuya, H., et al. 2003. Characterization of long cDNA clones from human adult spleen. II. The complete sequences of 81 cDNA clones. DNA Res. 10: 49-57.

CHROMOSOMAL LOCATION

Genetic locus: Afap1l1 (mouse) mapping to 18 E1.

PRODUCT

AFAP-1L1 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-1L1 shRNA Plasmid (m): sc-140895-SH and AFAP-1L1 shRNA (m) Lentiviral Particles: sc-140895-V as alternate gene silencing products.

For independent verification of AFAP-1L1 (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-140895A, sc-140895B and sc-140895C.

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-1L1 siRNA (m) is recommended for the inhibition of AFAP-1L1 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-1L1 (D-6): sc-514788 is recommended as a control antibody for monitoring of AFAP-1L1 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-1L1 gene expression knockdown using RT-PCR Primer: AFAP-1L1 (m)-PR: sc-140895-PR (20 µl). Annealing temperature for the primers should be 55-60° C and the extension temperature should be 68-72° C.

PROTOCOLS

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