

# AFAP-1L2 siRNA (h): sc-90824

## 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-1L2 (Actin filament-associated protein 1-like 2), also known as XB130, is a 818 amino acid cytoplasmic protein that contains two Pleckstrin homology (PH) domains, which are normally found in proteins involved in intracellular signaling. Like its relative AFAP-110, AFAP-1L2 interacts with Src kinase and may play a role in Src-regulated transcription activation. AFAP-1L2 is expressed in thyroid and spleen and can also be detected at lower levels in lung, brain, pancreas and kidney. There are four isoforms of AFAP-1L2 that are produced as a result of alternative splicing events.

## REFERENCES

1. Gatesman, A., et al. 2004. Protein kinase C $\alpha$  activates c-Src and induces podosome formation via AFAP-110. *Mol. Cell. Biol.* 24: 7578-7597.
2. Deloukas, P., et al. 2004. The DNA sequence and comparative analysis of human chromosome 10. *Nature* 429: 375-381.
3. Zhang, Y., et al. 2005. Time-resolved mass spectrometry of tyrosine phosphorylation sites in the epidermal growth factor receptor signaling network reveals dynamic modules. *Mol. Cell. Proteomics* 4: 1240-1250.
4. Rikova, K., et al. 2007. Global survey of phosphotyrosine signaling identifies oncogenic kinases in lung cancer. *Cell* 131: 1190-1203.

## CHROMOSOMAL LOCATION

Genetic locus: AFAP1L2 (human) mapping to 10q25.3.

## PRODUCT

AFAP-1L2 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  $\mu$ M solution once resuspended using protocol below. Suitable for 50-100 transfections. Also see AFAP-1L2 shRNA Plasmid (h): sc-90824-SH and AFAP-1L2 shRNA (h) Lentiviral Particles: sc-90824-V as alternate gene silencing products.

For independent verification of AFAP-1L2 (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-90824A, sc-90824B and sc-90824C.

## 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  $\mu$ l of the RNase-free water provided. Resuspension of the siRNA duplex in 330  $\mu$ l of RNase-free water makes a 10  $\mu$ M solution in a 10  $\mu$ M Tris-HCl, pH 8.0, 20 mM NaCl, 1 mM EDTA buffered solution.

## APPLICATIONS

AFAP-1L2 siRNA (h) is recommended for the inhibition of AFAP-1L2 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  $\mu$ M in 66  $\mu$ 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

AFAP-1L2 (12): sc-136176 is recommended as a control antibody for monitoring of AFAP-1L2 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 $\kappa$  BP-HRP: sc-516102 or m-IgG $\kappa$  BP-HRP (Cruz Marker): sc-516102-CM (dilution range: 1:1000-1:10000), Cruz Marker<sup>™</sup> Molecular Weight Standards: sc-2035, UltraCruz<sup>®</sup> Blocking Reagent: sc-516214 and Western Blotting Luminol Reagent: sc-2048. 2) Immunofluorescence: use m-IgG $\kappa$  BP-FITC: sc-516140 or m-IgG $\kappa$  BP-PE: sc-516141 (dilution range: 1:50-1:200) with UltraCruz<sup>®</sup> Mounting Medium: sc-24941 or UltraCruz<sup>®</sup> Hard-set Mounting Medium: sc-359850.

## RT-PCR REAGENTS

Semi-quantitative RT-PCR may be performed to monitor AFAP-1L2 gene expression knockdown using RT-PCR Primer: AFAP-1L2 (h)-PR: sc-90824-PR (20  $\mu$ l). Annealing temperature for the primers should be 55-60° C and the extension temperature should be 68-72° C.

## SELECT PRODUCT CITATIONS

1. Moodley, S., et al. 2016. Stimulus-dependent dissociation between XB130 and Tks5 scaffold proteins promotes airway epithelial cell migration. *Oncotarget* 7: 76437-76452.
2. Shen, J., et al. 2017. XB130 enhances invasion and migration of human colorectal cancer cells by promoting epithelial-mesenchymal transition. *Mol. Med. Rep.* 16: 5592-5598.

## RESEARCH USE

For research use only, not for use in diagnostic procedures.

## PROTOCOLS

See our web site at [www.scbt.com](http://www.scbt.com) for detailed protocols and support products.