SANTA CRUZ BIOTECHNOLOGY, INC.

Aftiphilin siRNA (h): sc-94965

BACKGROUND

Aftiphilin (AFTPH) is a 937 amino acid cytoplasmic protein involved in membrane trafficking. Aftiphilin is expressed predominantly in brain and colocalizes at synapses with synaptophysin and AP-2. Aftiphilin also colocalizes with γ1-Adaptin and Clathrin, and contains eight γ-ear-binding motifs, two WXXF-acidic motifs and three FXXFXXF/L motifs. Aftiphilin's WXXF motifs mediate the binding of accessory proteins to the ear domains of AP-1, AP-2 and GGAs via hydrophobic interactions. Aftiphilin interacts with the GAE domains of γ1-Adaptin, γ2-Adaptin, GGA1 and GGA3. Five Aftiphilin isoforms exist as a result of alternative splicing events, and Aftiphilin is encoded by a gene mapping to human chromosome 2p14. As the second largest human chromosome, chromosome 2 consists of 237 million bases, encodes over 1,400 genes and makes up approximately 8% of the human genome.

REFERENCES

1. Nakayama, K. and Wakatsuki, S. 2003. The structure and function of GGAs, the traffic controllers at the TGN sorting crossroads. Cell Struct. Funct. 28: 431-442.
2. Ritter, B., et al. 2004. Two WXXF-based motifs in NECAPs define the specificity of accessory protein binding to AP-1 and AP-2. EMBO J. 23: 3701-3710.
3. Mattera, R., et al. 2004. Definition of the consensus motif recognized by γ-Adaptin ear domains. J. Biol. Chem. 279: 8018-8028.
4. Burman, J.L., et al. 2005. Aftiphilin is a component of the clathrin machinery in neurons. FEBS Lett. 579: 2177-2184.
5. Hirst, J., et al. 2005. The Aftiphilin/p200/γ-synergin complex. Mol. Biol. Cell 16: 2554-2565.
6. Lui-Roberts, W.W., et al. 2008. Aftiphilin and γ-synergin are required for secretagogue sensitivity of Weibel-Palade bodies in endothelial cells. Mol. Biol. Cell 19: 5072-5081.

CHROMOSOMAL LOCATION

Genetic locus: AFTPH (human) mapping to 2p14.

PRODUCT

Aftiphilin 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 µM solution once resuspended using protocol below. Suitable for 50-100 transfections. Also see Aftiphilin shRNA Plasmid (h): sc-94965-SH and Aftiphilin shRNA (h) Lentiviral Particles: sc-94965-V as alternate gene silencing products.

For independent verification of Aftiphilin (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-94965A, sc-94965B and sc-94965C.

PROTOCOLS

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

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

Aftiphilin siRNA (h) is recommended for the inhibition of Aftiphilin 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 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.

RT-PCR REAGENTS

Semi-quantitative RT-PCR may be performed to monitor Aftiphilin gene expression knockdown using RT-PCR Primer: Aftiphilin (h)-PR: sc-94965-PR (20 µl, 554 bp). Annealing temperature for the primers should be 55-60° C and the extension temperature should be 68-72° C.

SELECT PRODUCT CITATIONS

1. Law, I.K., et al. 2015. Neurotensin—regulated miR-133α is involved in proinflammatory signalling in human colonic epithelial cells and in experimental colitis. Gut 64: 1095-1104.

RESEARCH USE

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