

Amphiphysin II siRNA (h): sc-29804

BACKGROUND

Amphiphysin is a brain-enriched protein that exhibits N-terminal lipid interaction and functions as a dimer. Amphiphysin contains a membrane bending BAR domain, a middle Clathrin and adaptor binding domain and a C-terminal SH3 domain. In the brain, Amphiphysin I and II form heterodimers that bind to the Clathrin associated GTPase Dynamin via their SH3 domains. This association is essential for synaptic vesicle recycling in neurons, as it precedes the binding of Dynamin to the Clathrin-coated pits and the subsequent vesicle budding. In other tissues, Amphiphysin may play a key role in other membrane bending and curvature stabilization events. The mammalian Amphiphysins, Amphiphysin I and Amphiphysin II, have similar overall structure. An ubiquitous splice form of Amphiphysin II that does not contain Clathrin or adaptor interactions is highly expressed in muscle tissue and is involved in the formation and stabilization of the T tubule network.

REFERENCES

1. Lichte, B., et al. 1992. Amphiphysin, a novel protein associated with synaptic vesicles. *EMBO J.* 11: 2521-2530.
2. Yamamoto, R., et al. 1995. Primary structure of human Amphiphysin, the dominant autoantigen of paraneoplastic stiff-man syndrome, and mapping of its gene (AMPH) to chromosome 7p13-p14. *Hum. Mol. Genet.* 4: 265-268.

CHROMOSOMAL LOCATION

Genetic locus: BIN1 (human) mapping to 2q14.3.

PRODUCT

Amphiphysin II 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 μ M solution once resuspended using protocol below. Suitable for 50-100 transfections. Also see Amphiphysin II shRNA Plasmid (h): sc-29804-SH and Amphiphysin II shRNA (h) Lentiviral Particles: sc-29804-V as alternate gene silencing products.

For independent verification of Amphiphysin II (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-29804A, sc-29804B and sc-29804C.

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

APPLICATIONS

Amphiphysin II siRNA (h) is recommended for the inhibition of Amphiphysin II 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 μ M in 66 μ 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

Amphiphysin II (2F11): sc-23918 is recommended as a control antibody for monitoring of Amphiphysin II 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 κ BP-HRP: sc-516102 or m-IgG κ 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-IgG κ BP-FITC: sc-516140 or m-IgG κ 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 Amphiphysin II gene expression knockdown using RT-PCR Primer: Amphiphysin II (h)-PR: sc-29804-PR (20 μ l, 454 bp). Annealing temperature for the primers should be 55-60° C and the extension temperature should be 68-72° C.

SELECT PRODUCT CITATIONS

1. Kinney, E.L., et al. 2008. Adenovirus E1A oncoprotein liberates c-Myc activity to promote cell proliferation through abating BIN1 expression via an Rb/E2F1-dependent mechanism. *J. Cell. Physiol.* 216: 621-631.
2. Cassimere, E.K., et al. 2009. The c-Myc-interacting proapoptotic tumor suppressor BIN1 is a transcriptional target for E2F1 in response to DNA damage. *Cell Death Differ.* 16: 1641-1653.
3. Pyndiah, S., et al. 2011. c-Myc suppresses BIN1 to release poly(ADP-ribose) polymerase 1: a mechanism by which cancer cells acquire cisplatin resistance. *Sci. Signal.* 4: ra19.
4. Goonawardane, N., et al. 2017. Phosphorylation of serine 225 in hepatitis C virus NS5A regulates protein-protein interactions. *J. Virol.* 91: e00805-17.

RESEARCH USE

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