

APPL2 siRNA (h): sc-61982

BACKGROUND

The APPL family of proteins are involved in linking, trafficking and signaling downstream of tyrosine kinase receptors. APPL1, also designated adaptor protein containing pH domain, PTB domain and leucine zipper motif 1; APPL; or DCC interacting protein 13 α (DIP13 α), and APPL2, also designated adaptor protein containing pH domain, PTB domain and leucine zipper motif 2 or DCC interacting protein 13 β (DIP13 β), are involved in the coupling of epidermal growth factor (EGF) signaling and chromatin remodeling in the nucleus. They associate with GTPase Rab 5 and are released from the plasma membrane and translocated to the nucleus. In the nucleus, APPL1 and APPL2 associate with NuRD/MeCP1 and are essential for cell growth and proliferation. APPL2 also associates with follicle stimulating hormone receptor (FSHR). APPL2 is highly expressed in heart, brain, skeletal muscle, and kidney. APPL2 shares 54% homology with APPL1.

REFERENCES

1. Nechamen, C.A., et al. 2004. Human follicle-stimulating hormone (FSH) receptor interacts with the adaptor protein APPL1 in HEK 293 cells: potential involvement of the PI3K pathway in FSH signaling. *Biol. Reprod.* 71: 629-636.
2. Habermann, B. 2004. The BAR-domain family of proteins: a case of bending and binding? *EMBO Rep.* 5: 250-255.
3. Miaczynska, M., et al. 2004. APPL proteins link Rab5 to nuclear signal transduction via an endosomal compartment. *Cell* 116: 445-456.
4. Mao, X., et al. 2006. APPL1 binds to adiponectin receptors and mediates adiponectin signalling and function. *Nat. Cell Biol.* 8: 516-523.
5. Lo, H.W., et al. 2006. Nuclear-cytoplasmic transport of EGFR involves receptor endocytosis, importin β 1 and CRM1. *J. Cell. Biochem.* 98: 1570-1583.
6. Varsano, T., et al. 2006. GIPC is recruited by APPL to peripheral TrkA endosomes and regulates TrkA trafficking and signaling. *Mol. Cell. Biol.* 26: 8942-8952.

CHROMOSOMAL LOCATION

Genetic locus: APPL2 (human) mapping to 12q23.3.

PRODUCT

APPL2 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 APPL2 shRNA Plasmid (h): sc-61982-SH and APPL2 shRNA (h) Lentiviral Particles: sc-61982-V as alternate gene silencing products.

For independent verification of APPL2 (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-61982A, sc-61982B and sc-61982C.

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 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

APPL2 siRNA (h) is recommended for the inhibition of APPL2 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

APPL2 (F-2): sc-398859 is recommended as a control antibody for monitoring of APPL2 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 APPL2 gene expression knockdown using RT-PCR Primer: APPL2 (h)-PR: sc-61982-PR (20 μ l). 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.