

AP-4 ϵ siRNA (h): sc-105078

BACKGROUND

AP-4 (adaptor-related protein complex 4) is a heterotetrameric complex comprised of subunits designated AP-4 β , AP-4 ϵ , AP-4 μ and AP-4 σ . AP-4 mediates the incorporation of cargo into transport vesicles by interacting with motifs present in the cytoplasmic tails of their specific cargo proteins at different intracellular locations. AP-4 localizes on the cytoplasmic face of the *trans*-Golgi network (TGN), Clathrin coat machinery of endosomes, and transport vesicles. AP-4 can position together with the Cl-MPR (cation-independent mannose 6-phosphate receptor). AP-4 may influence trafficking of glutamate receptor $\delta 2$ (Grid2) in the brain. AP-4 participates in basolateral sorting in epithelial cells. AP-4 complex is expressed ubiquitously in many regions of brain, with localization on the Golgi-like structures in the cell bodies and dendrites of neurons.

REFERENCES

1. Hirst, J., et al. 1999. Characterization of a fourth adaptor-related protein complex. *Mol. Biol. Cell* 10: 2787-2802.
2. Dell'Angelica, E.C., et al. 1999. AP-4, a novel protein complex related to Clathrin adaptors. *J. Biol. Chem.* 274: 7278-7285.
3. Boehm, M., et al. 2001. Functional and physical interactions of the adaptor protein complex AP-4 with ADP-ribosylation factors (ARFs). *EMBO J.* 20: 6265-6276.
4. Aguilar, R.C., et al. 2001. Signal-binding specificity of the $\mu 4$ subunit of the adaptor protein complex AP-4. *J. Biol. Chem.* 276: 13145-13152.
5. Simmen, T., et al. 2002. AP-4 binds basolateral signals and participates in basolateral sorting in epithelial MDCK cells. *Nat. Cell Biol.* 4: 154-159.
6. Yap, C.C., et al. 2003. Adaptor protein complex-4 (AP-4) is expressed in the central nervous system neurons and interacts with glutamate receptor $\delta 2$. *Mol. Cell. Neurosci.* 24: 283-295.
7. Barois, N., et al. 2005. The adaptor protein AP-4 as a component of the Clathrin coat machinery: a morphological study. *Biochem. J.* 385: 503-510.

CHROMOSOMAL LOCATION

Genetic locus: AP4E1 (human) mapping to 15q21.2.

PRODUCT

AP-4 ϵ 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 AP-4 ϵ shRNA Plasmid (h): sc-105078-SH and AP-4 ϵ shRNA (h) Lentiviral Particles: sc-105078-V as alternate gene silencing products.

For independent verification of AP-4 ϵ (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-105078A, sc-105078B and sc-105078C.

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

AP-4 ϵ siRNA (h) is recommended for the inhibition of AP-4 ϵ 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

AP-4 ϵ (32): sc-135835 is recommended as a control antibody for monitoring of AP-4 ϵ 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 AP-4 ϵ gene expression knockdown using RT-PCR Primer: AP-4 ϵ (h)-PR: sc-105078-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.