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AP-4 μ shRNA (h) Lentiviral Particles: sc-43616-V

BACKGROUND

AP-4 (adapter-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 CI-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

- Hirst, J., et al. 1999. Characterization of a fourth adaptor-related protein complex. *Mol. Biol. Cell.* 10: 2787-802.
- Dell'Angelica, E.C., et al. 1999. AP-4, a novel protein complex related to Clathrin adaptors. *J. Biol. Chem.* 274: 7278-7285.
- 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.
- 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.
- 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.

CHROMOSOMAL LOCATION

Genetic locus: AP4M1 (human) mapping to 7q22.1.

PRODUCT

AP-4 μ shRNA (h) Lentiviral Particles is a pool of concentrated, transduction-ready viral particles containing 3 target-specific constructs that encode 19-25 nt (plus hairpin) shRNA designed to knock down gene expression. Each vial contains 200 μ l frozen stock containing 1.0×10^6 infectious units of virus (IFU) in Dulbecco's Modified Eagle's Medium with 25 mM HEPES pH 7.3. Suitable for 10-20 transductions. Also see AP-4 μ siRNA (h): sc-43616 and AP-4 μ shRNA Plasmid (h): sc-43616-SH as alternate gene silencing products.

STORAGE

Store lentiviral particles at -80°C . Stable for at least one year from the date of shipment. Once thawed, particles can be stored at 4°C for up to one week. Avoid repeated freeze thaw cycles.

PROTOCOLS

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

APPLICATIONS

AP-4 μ shRNA (h) Lentiviral Particles is recommended for the inhibition of AP-4 μ expression in human cells.

SUPPORT REAGENTS

Control shRNA Lentiviral Particles: sc-108080. Available as 200 μ l frozen viral stock containing 1.0×10^6 infectious units of virus (IFU); contains an shRNA construct encoding a scrambled sequence that will not lead to the specific degradation of any known cellular mRNA.

GENE EXPRESSION MONITORING

AP-4 μ (G-20): sc-18488 is recommended as a control antibody for monitoring of AP-4 μ 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 (secondary) reagents are recommended: 1) Western Blotting: use donkey anti-goat IgG-HRP: sc-2020 (dilution range: 1:2000-1:100,000) or Cruz Marker™ compatible donkey anti-goat IgG-HRP: sc-2033 (dilution range: 1:2000-1:5000), Cruz Marker™ Molecular Weight Standards: sc-2035, TBS Blotto A Blocking Reagent: sc-2333 and Western Blotting Luminol Reagent: sc-2048. 2) Immunofluorescence: use donkey anti-goat IgG-FITC: sc-2024 (dilution range: 1:100-1:400) or donkey anti-goat IgG-TR: sc-2783 (dilution range: 1:100-1:400) with UltraCruz™ Mounting Medium: sc-24941.

RT-PCR REAGENTS

Semi-quantitative RT-PCR may be performed to monitor AP-4 μ gene expression knockdown using RT-PCR Primer: AP-4 μ (h)-PR: sc-43616-PR (20 μ l). Annealing temperature for the primers should be $55-60^\circ\text{C}$ and the extension temperature should be $68-72^\circ\text{C}$.

BIOSAFETY

Lentiviral particles can be employed in standard Biosafety Level 2 tissue culture facilities (and should be treated with the same level of caution as with any other potentially infectious reagent). Lentiviral particles are replication-incompetent and are designed to self-inactivate after transduction and integration of shRNA constructs into genomic DNA of target cells.

RESEARCH USE

The purchase of this product conveys to the buyer the nontransferable right to use the purchased amount of the product and all replicates and derivatives for research purposes conducted by the buyer in his laboratory only (whether the buyer is an academic or for-profit entity). The buyer cannot sell or otherwise transfer (a) this product (b) its components or (c) materials made using this product or its components to a third party, or otherwise use this product or its components or materials made using this product or its components for Commercial Purposes.