SANTA CRUZ BIOTECHNOLOGY, INC.

AP-2μ1 siRNA (m): sc-60185

BACKGROUND

Adaptins are heterotetrameric subunits of adaptors, which are complexes involved in the formation of Clathrin-coated pits for vesicle-mediated endocytosis. Clathrin and its associated heterotetrameric protein complexes make up the main protein components of the coat surrounding the cytoplasmic face of coated vesicles. The Adaptin family, comprising α, β, β' and γ classes, is also responsible for the transport of ligand-receptor complexes from plasma membranes and the *trans*-Golgi network to lysosomes. Two main types of adaptor proteins (APs), AP-1 and AP-2, are found in Clathrin-coated structures located at the Golgi complex and the plasma membrane of mammalian cells, respectively. Adaptor protein complex 2 (AP-2) is composed of two large Adaptins (α1A/AP2A1 and β1/AP2B1), a medium Adaptin (μ2/AP-2μ1) and a small Adaptin (σ2 long/AP2S1). AP-2μ1, a 435 amino acid protein, links Clathrin to receptors in coated vesicles.

REFERENCES

1. Takatsu, H., et al. 1998. Identification and characterization of novel clathrin adaptor-related proteins. J. Biol. Chem. 273: 24693-24700.
2. Nakatsu, F., et al. 1999. Genomic structure and chromosome mapping of the genes encoding clathrin-associated adaptor medium chains μ1A (AP1M1) and μ1B (AP1M2). Cytogenet. Cell Genet. 87: 53-58.
3. Shim, J., et al. 2000. Distinct and redundant functions of μ1 medium chains of the AP-1 clathrin-associated protein complex in the nematode *Caenorhabditis elegans*. Mol. Biol. Cell 11: 2743-2756.
4. Boehm, M., et al. 2001. Adaptins: the final recount. Mol. Biol. Cell 12: 2907-2920.
5. Takatsu, H., et al. 2001. Similar subunit interactions contribute to assembly of clathrin adaptor complexes and COPI complex: analysis using yeast three-hybrid system. Biochem. Biophys. Res. Commun. 284: 1083-1089.
6. Kierczak, M., et al. 2003. Role of the adaptins, Dynamin-like GTPases and Rab proteins in metabolic disorders and various infections. Postepy Hig. Med. Dosw. 57: 727-737.

CHROMOSOMAL LOCATION

Genetic locus: Ap2m1 (mouse) mapping to 16 A3.

PRODUCT

AP-2μ1 siRNA (m) 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 AP-2μ1 shRNA Plasmid (m): sc-60185-SH and AP-2μ1 shRNA (m) Lentiviral Particles: sc-60185-V as alternate gene silencing products.

For independent verification of AP-2μ1 (m) gene silencing results, we also provide the individual siRNA duplex components. Each is available as 3.3 nmol of lyophilized siRNA. These include: sc-60185A, sc-60185B and sc-60185C.

RESEARCH USE

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

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

AP-2μ1 siRNA (m) is recommended for the inhibition of AP-2μ1 expression in mouse 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.

GENE EXPRESSION MONITORING

AP-2μ1 (D-7): sc-515920 is recommended as a control antibody for monitoring of AP-2μ1 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-IgGκ BP-HRP: sc-516102 or m-IgGκ 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-IgGκ BP-FITC: sc-516140 or m-IgGκ 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-2μ1 gene expression knockdown using RT-PCR Primer: AP-2μ1 (m)-PR: sc-60185-PR (20 µl). Annealing temperature for the primers should be 55-60° C and the extension temperature should be 68-72° C.

PROTOCOLS

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