SANTA CRUZ BIOTECHNOLOGY, INC.

AP-2σ1 siRNA (h): sc-97710

BACKGROUND

Adapter-related protein complex 2 (AP-2) is one of the two major clathrin-associated adaptor complexes that links clathrin to receptors in coated vesicles. It interacts with the cytoplasmic tail of membrane proteins, thereby leading to their selection and concentration. This complex has also been shown to bind polyphosphoinositides. Existing as a heterotetramer, AP-2 is comprised of two large chains, a medium chain and a small chain. Adapter-related protein complex 2 subunit σ-1 (AP-2σ1), also known as clathrin coat assembly protein AP17 or σ2-adaptin, is the 142 amino acid protein small chain of the AP-2 complex. Localized to the cytoplasmic side of the cell membrane, two named isoforms of AP-2σ1 exist as a result of alternative splicing events.

REFERENCES

1. Kirchhausen, T., et al. 1991. AP17 and AP19, the mammalian small chains of the clathrin-associated protein complexes show homology to Yap17p, their putative homolog in yeast. J. Biol. Chem. 266: 11153-11157.
2. Brodsky, F.M., et al. 1991. Clathrin light chains: arrays of protein motifs that regulate coated-vesicle dynamics. Trends Biochem. Sci. 16: 208-213.
3. Pley, U., et al. 1993. Clathrin: its role in receptor-mediated vesicular transport and specialized functions in neurons. Crit. Rev. Biochem. Mol. Biol. 28: 431-464.
4. Winterpacht, A., et al. 1996. Human CLAPS2 encoding AP17, a small chain of the clathrin-associated protein complex: cDNA cloning and chromosomal assignment to 19q13.2→q13.3. Cytogenet. Cell Genet. 75: 132-135.
5. Holzmann, K., et al. 1998. A novel spliced transcript of human CLAPS2 encoding a protein alternative to clathrin adaptor protein AP17. Gene 220: 39-44.
6. Online Mendelian Inheritance in Man, OMIM™. 2001. Johns Hopkins University, Baltimore, MD. MIM Number: 602242. World Wide Web URL: http://www.ncbi.nlm.nih.gov/omim/
7. Daub, H., et al. 2008. Kinase-selective enrichment enables quantitative phosphoproteomics of the kinome across the cell cycle. Mol. Cell 31: 438-448.

CHROMOSOMAL LOCATION

Genetic locus: AP2S1 (human) mapping to 19q13.32.

PRODUCT

AP-2σ1 siRNA (h) is a pool of 2 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-2σ1 shRNA Plasmid (h): sc-97710-SH and AP-2σ1 shRNA (h) Lentiviral Particles: sc-97710-V as alternate gene silencing products.

For independent verification of AP-2σ1 (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-97710A and sc-97710B.

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-2σ1 siRNA (h) is recommended for the inhibition of AP-2σ1 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 µM in 60 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-2σ1 (5-RY21): sc-134261 is recommended as a control antibody for monitoring of AP-2σ1 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-2σ1 gene expression knockdown using RT-PCR Primer: AP-2σ1 (h)-PR: sc-97710-PR (20 µl). 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.

PROTOCOLS

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