

AP-180 siRNA (h): sc-29698

BACKGROUND

Clathrin-coated pits and vesicles are assembled for receptor-mediated endocytosis through interaction with Clathrin associated protein complexes. Vesicle transport is mediated from the *trans*-Golgi network by the adapter complex AP-1 and from the plasma membrane by the AP-2 complex. The AP-1 and AP-2 adapter protein complexes consist of Clathrin binding Adaptin proteins. AP-180, also known as SNAP91 (synaptosomal-associated protein, 91kDa homolog (mouse)) or CALM, is a 907 amino acid cell membrane protein that contains an ENTH (epsin N-terminal homology) domain. AP-180 binds to Clathrin triskelion via its N-terminal clathrin binding site, inducing assembly into 60-70 nm coats. Existing as three alternatively spliced isoforms, the gene encoding AP-180 maps to human chromosome 6q14.2 and mouse chromosome 9 E3.1.

REFERENCES

1. Robinson, M.S. 1989. Cloning of cDNAs encoding two related 100 kDa coated vesicle proteins (α -adaptins). *J. Cell Biol.* 108: 833-842.
2. Kirchhausen, T., et al. 1989. Structural and functional division into two domains of the large (100 to 115 kDa) chains of the clathrin-associated protein complex AP-2. *Proc. Natl. Acad. Sci. USA* 86: 2612-2616.
3. Robinson, M.S. 1990. Cloning and expression of γ -adaptin, a component of clathrin-coated vesicles associated with the Golgi apparatus. *J. Cell Biol.* 111: 2319-2326.
4. Ponnambalam, S., et al. 1990. Conservation and diversity in families of coated vesicle adaptins. *J. Biol. Chem.* 265: 4814-4820.
5. Morris, S.A., et al. 1993. Clathrin assembly protein AP180: primary structure, domain organization and identification of a clathrin binding site. *EMBO J.* 12: 667-675.
6. Ball, C.L., et al. 1995. Expression and localization of α -adaptin isoforms. *J. Cell Sci.* 108: 2865-2875.
7. Mellman, I. 1996. Endocytosis and molecular sorting. *Annu. Rev. Cell Dev. Biol.* 12: 575-625.

CHROMOSOMAL LOCATION

Genetic locus: SNAP91 (human) mapping to 6q14.2.

PRODUCT

AP-180 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-180 shRNA Plasmid (h): sc-29698-SH and AP-180 shRNA (h) Lentiviral Particles: sc-29698-V as alternate gene silencing products.

For independent verification of AP-180 (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-29698A, sc-29698B and sc-29698C.

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 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-180 siRNA (h) is recommended for the inhibition of AP-180 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-180 (AP180-I): sc-58229 is recommended as a control antibody for monitoring of AP-180 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-180 gene expression knockdown using RT-PCR Primer: AP-180 (h)-PR: sc-29698-PR (20 μ l). 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.