

# AP-3 $\sigma$ siRNA (h): sc-60180

## 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. AP-3 (also designated AP180 or F1-20) is a synapse-specific Clathrin assembly protein. The protein CALM (Clathrin assembly protein lymphoid myeloid leukemia) is highly homologous to AP180 and may also be involved in Clathrin assembly. AP-3 $\delta$ , AP-3 $\alpha$  and AP-3 $\mu$  are important parts of the AP-3 complex.

## REFERENCES

1. Robinson, M.S. 1989. Cloning of cDNAs encoding two related 100-kD coated vesicle proteins ( $\alpha$ -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  $\gamma$ -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. Simpson, F., et al. 1997. Characterization of the adaptor-related protein complex, AP-3. *J. Cell Biol.* 137: 835-845.
6. Lefrancois, S., et al. 2004. An ear-core interaction regulates the recruitment of the AP-3 complex to membranes. *Dev. Cell* 7: 619-625.
7. Dong, X., et al. 2005. AP-3 directs the intracellular trafficking of HIV-1 Gag and plays a key role in particle assembly. *Cell* 120: 663-674.
8. Coleman, S.H., et al. 2005. Leucine-specific, functional interactions between human immunodeficiency virus type 1 Nef and adaptor protein complexes. *J. Virol.* 79: 2066-2078.

## CHROMOSOMAL LOCATION

Genetic locus: AP3S1 (human) mapping to 5q22.3.

## PRODUCT

AP-3 $\sigma$  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  $\mu$ M solution once resuspended using protocol below. Suitable for 50-100 transfections. Also see AP-3 $\sigma$  shRNA Plasmid (h): sc-60180-SH and AP-3 $\sigma$  shRNA (h) Lentiviral Particles: sc-60180-V as alternate gene silencing products.

For independent verification of AP-3 $\sigma$  (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-60180A, sc-60180B and sc-60180C.

## PROTOCOLS

See our web site at [www.scbt.com](http://www.scbt.com) for detailed protocols and support products.

## STORAGE AND RESUSPENSION

Store lyophilized siRNA duplex at -20 $^{\circ}$  C with desiccant. Stable for at least one year from the date of shipment. Once resuspended, store at -20 $^{\circ}$  C, avoid contact with RNAses and repeated freeze thaw cycles.

Resuspend lyophilized siRNA duplex in 330  $\mu$ l of the RNase-free water provided. Resuspension of the siRNA duplex in 330  $\mu$ l of RNase-free water makes a 10  $\mu$ M solution in a 10  $\mu$ M Tris-HCl, pH 8.0, 20 mM NaCl, 1 mM EDTA buffered solution.

## APPLICATIONS

AP-3 $\sigma$  siRNA (h) is recommended for the inhibition of AP-3 $\sigma$  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  $\mu$ M in 66  $\mu$ 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-3 $\sigma$  (6): sc-136338 is recommended as a control antibody for monitoring of AP-3 $\sigma$  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 $\kappa$  BP-HRP: sc-516102 or m-IgG $\kappa$  BP-HRP (Cruz Marker): sc-516102-CM (dilution range: 1:1000-1:10000), Cruz Marker<sup>™</sup> Molecular Weight Standards: sc-2035, UltraCruz<sup>®</sup> Blocking Reagent: sc-516214 and Western Blotting Luminol Reagent: sc-2048. 2) Immunofluorescence: use m-IgG $\kappa$  BP-FITC: sc-516140 or m-IgG $\kappa$  BP-PE: sc-516141 (dilution range: 1:50-1:200) with UltraCruz<sup>®</sup> Mounting Medium: sc-24941 or UltraCruz<sup>®</sup> Hard-set Mounting Medium: sc-359850.

## RT-PCR REAGENTS

Semi-quantitative RT-PCR may be performed to monitor AP-3 $\sigma$  gene expression knockdown using RT-PCR Primer: AP-3 $\sigma$  (h)-PR: sc-60180-PR (20  $\mu$ l). Annealing temperature for the primers should be 55-60 $^{\circ}$  C and the extension temperature should be 68-72 $^{\circ}$  C.

## RESEARCH USE

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