

# AP-2 $\mu$ 1 siRNA (h): sc-60184

## 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  $\alpha$ ,  $\beta$ ,  $\beta'$  and  $\gamma$  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 ( $\alpha$ 1A/AP2A1 and  $\beta$ 1/AP2B1), a medium Adaptin ( $\mu$ 2/AP-2 $\mu$ 1) and a small Adaptin ( $\sigma$ 2 long/AP2S1). AP-2 $\mu$ 1, 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  $\mu$ 1A (AP1M1) and  $\mu$ 1B (AP1M2). *Cytogenet. Cell Genet.* 87: 53-58.
3. Shim, J., et al. 2000. Distinct and redundant functions of  $\mu$ 1 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 (human) mapping to 3q27.1.

## PRODUCT

AP-2 $\mu$ 1 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-2 $\mu$ 1 shRNA Plasmid (h): sc-60184-SH and AP-2 $\mu$ 1 shRNA (h) Lentiviral Particles: sc-60184-V as alternate gene silencing products.

For independent verification of AP-2 $\mu$ 1 (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-60184A, sc-60184B and sc-60184C.

## 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  $\mu$ 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-2 $\mu$ 1 siRNA (h) is recommended for the inhibition of AP-2 $\mu$ 1 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-2 $\mu$ 1 (D-7): sc-515920 is recommended as a control antibody for monitoring of AP-2 $\mu$ 1 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™ Molecular Weight Standards: sc-2035, UltraCruz® 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® 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-2 $\mu$ 1 gene expression knockdown using RT-PCR Primer: AP-2 $\mu$ 1 (h)-PR: sc-60184-PR (20  $\mu$ 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](http://www.scbt.com) for detailed protocols and support products.