

# AP-3 $\beta$ siRNA (m): sc-41166

## BACKGROUND

The widely expressed adaptor-like complex AP-3 is involved in protein sorting in exocytic/endocytic pathways and is composed of four distinct subunits. One of these subunits, AP-3 $\beta$ , also known as  $\beta$ 3A-Adaptin, is closely related to the neuron-specific protein  $\beta$ -NAP and shares 61% overall identity.  $\beta$ -NAP (also known as  $\beta$ -3B-Adaptin) is a homolog of the  $\beta/\beta'$ -Adaptins.  $\beta$ -NAP is related to one of the adaptor subunits of Clathrin-coated vesicles and is also part of an adaptor-like complex which is not associated with Clathrin. Casein kinase I selectively phosphorylates the AP-3 $\beta$  and  $\beta$ -NAP subunits at its hinge domain; inhibiting the kinase hinders the recruitment of AP-3 to synaptic vesicles.

## REFERENCES

1. Simpson, F., et al. 1996. A novel adaptor-related protein complex. *J. Cell Biol.* 133: 749-760.
2. Dell'Angelica, E.C., et al. 1997.  $\beta$ -3A-Adaptin, a subunit of the adaptor-like complex AP-3. *J. Biol. Chem.* 272: 15078-15084.
3. Dell'Angelica, E.C., et al. 1997. AP-3: an adaptor-like protein complex with ubiquitous expression. *EMBO J.* 16: 917-928.
4. Simpson, F., et al. 1997. Characterization of the adaptor-related protein complex, AP-3. *J. Cell Biol.* 137: 835-845.
5. Dell'Angelica, E.C., et al. 1998. Association of the AP-3 adaptor complex with Clathrin. *Science* 280: 431-434.
6. Mullins, C., et al. 2000. Distinct requirements for the AP-3 adaptor complex in pigment granule and synaptic vesicle biogenesis in *Drosophila melanogaster*. *Mol. Gen. Genet.* 263: 1003-1014.
7. Faundez, V.V. and Kelly, R.B. 2000. The AP-3 complex required for endosomal synaptic vesicle biogenesis is associated with a casein kinase I  $\alpha$ -like isoform. *Mol. Biol. Cell* 11: 2591-2604.

## CHROMOSOMAL LOCATION

Genetic locus: Ap3b1 (mouse) mapping to 13 D1.

## PRODUCT

AP-3 $\beta$  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  $\mu$ M solution once resuspended using protocol below. Suitable for 50-100 transfections. Also see AP-3 $\beta$  shRNA Plasmid (m): sc-41166-SH and AP-3 $\beta$  shRNA (m) Lentiviral Particles: sc-41166-V as alternate gene silencing products.

For independent verification of AP-3 $\beta$  (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-41166A, sc-41166B and sc-41166C.

## 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° 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-3 $\beta$  siRNA (m) is recommended for the inhibition of AP-3 $\beta$  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  $\mu$ 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 $\beta$  (3B4): sc-517083 is recommended as a control antibody for monitoring of AP-3 $\beta$  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-3 $\beta$  gene expression knockdown using RT-PCR Primer: AP-3 $\beta$  (m)-PR: sc-41166-PR (20  $\mu$ l). 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.