

# $\gamma$ 1-Adaptin siRNA (h): sc-29578

## 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 ( $\gamma$  and  $\beta$ 1 for AP-1,  $\alpha$  and  $\beta$ 2 for AP-2) and two smaller subunits known as AP50 and AP17. The  $\alpha$  and  $\beta$  Adaptin chains have a similar two-domain organization with C-terminal domains that vary in both sequence and length.  $\alpha$ -Adaptin splice variants A and C display variable relative expression levels and differential distribution in different tissues. AP180 (also designated AP-3 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.

## REFERENCES

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- 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.
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- Ponnambalam, S., et al. 1990. Conservation and diversity in families of coated vesicle Adaptins. *J. Biol. Chem.* 265: 4814-4820.
- 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.
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- Dreyling, M.H., et al. 1996. The t(10;11)(p13;q14) in the U937 cell line results in the fusion of the AF10 gene and CALM, encoding a new member of the AP-3 Clathrin assembly protein family. *Proc. Natl. Acad. Sci. USA* 93: 4804-4809.

## CHROMOSOMAL LOCATION

Genetic locus: AP1G1 (human) mapping to 16q22.2.

## PRODUCT

$\gamma$ 1-Adaptin 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  $\gamma$ 1-Adaptin shRNA Plasmid (h): sc-29578-SH and  $\gamma$ 1-Adaptin shRNA (h) Lentiviral Particles: sc-29578-V as alternate gene silencing products.

For independent verification of  $\gamma$ 1-Adaptin (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-29578A, sc-29578B and sc-29578C.

## 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

$\gamma$ 1-Adaptin siRNA (h) is recommended for the inhibition of  $\gamma$ 1-Adaptin 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

$\gamma$ 1-Adaptin (F-10): sc-398867 is recommended as a control antibody for monitoring of  $\gamma$ 1-Adaptin 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  $\gamma$ 1-Adaptin gene expression knockdown using RT-PCR Primer:  $\gamma$ 1-Adaptin (h)-PR: sc-29578-PR (20  $\mu$ l, 453 bp). 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](http://www.scbt.com) for detailed protocols and support products.