

γ 1-Adaptin siRNA (m): sc-29579

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 (γ and β 1 for AP-1, α and β 2 for AP-2) and two smaller subunits known as AP50 and AP17. The α and β Adaptin chains have a similar two-domain organization with C-terminal domains that vary in both sequence and length. α -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

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. 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 (mouse) mapping to 8 D3.

PRODUCT

γ 1-Adaptin 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 μ M solution once resuspended using protocol below. Suitable for 50-100 transfections. Also see γ 1-Adaptin shRNA Plasmid (m): sc-29579-SH and γ 1-Adaptin shRNA (m) Lentiviral Particles: sc-29579-V as alternate gene silencing products.

For independent verification of γ 1-Adaptin (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-29579A, sc-29579B and sc-29579C.

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

γ 1-Adaptin siRNA (m) is recommended for the inhibition of γ 1-Adaptin 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 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

γ 1-Adaptin (F-10): sc-398867 is recommended as a control antibody for monitoring of γ 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 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 γ 1-Adaptin gene expression knockdown using RT-PCR Primer: γ 1-Adaptin (m)-PR: sc-29579-PR (20 μ 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.

PROTOCOLS

See our web site at www.scbt.com for detailed protocols and support products.