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α -Adaptin 2 shRNA (h) Lentiviral Particles: sc-43505-V

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 -Adaptin and β -Adaptin for AP-1; α -Adaptin 1, α -Adaptin 2 and β 2-Adaptin 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 1 and 2 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. 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.
2. Robinson, M.S. 1989. Cloning of cDNAs encoding two related 100-kD coated vesicle proteins (α -Adaptins). *J. Cell Biol.* 108: 833-842.
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.

CHROMOSOMAL LOCATION

Genetic locus: AP2A2 (human) mapping to 11p15.5.

PRODUCT

α -Adaptin 2 shRNA (h) Lentiviral Particles is a pool of concentrated, transduction-ready viral particles containing 3 target-specific constructs that encode 19-25 nt (plus hairpin) shRNA designed to knock down gene expression. Each vial contains 200 μ l frozen stock containing 1.0×10^6 infectious units of virus (IFU) in Dulbecco's Modified Eagle's Medium with 25 mM HEPES pH 7.3. Suitable for 10-20 transductions. Also see α -Adaptin 2 siRNA (h): sc-43505 and α -Adaptin 2 shRNA Plasmid (h): sc-43505-SH as alternate gene silencing products.

STORAGE

Store lentiviral particles at -80°C . Stable for at least one year from the date of shipment. Once thawed, particles can be stored at 4°C for up to one week. Avoid repeated freeze thaw cycles.

PROTOCOLS

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

APPLICATIONS

α -Adaptin 2 shRNA (h) Lentiviral Particles is recommended for the inhibition of α -Adaptin 2 expression in human cells.

SUPPORT REAGENTS

Control shRNA Lentiviral Particles: sc-108080. Available as 200 μ l frozen viral stock containing 1.0×10^6 infectious units of virus (IFU); contains an shRNA construct encoding a scrambled sequence that will not lead to the specific degradation of any known cellular mRNA.

GENE EXPRESSION MONITORING

α -Adaptin 2 (A-8): sc-377376 is recommended as a control antibody for monitoring of α -Adaptin 2 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 (secondary) reagents are recommended: 1) Western Blotting: use goat anti-mouse IgG-HRP: sc-2005 (dilution range: 1:2000-1:32,000) or Cruz Marker™ compatible goat anti-mouse IgG-HRP: sc-2031 (dilution range: 1:2000-1:5000), Cruz Marker™ Molecular Weight Standards: sc-2035, TBS Blotto A Blocking Reagent: sc-2333 and Western Blotting Luminol Reagent: sc-2048. 2) Immunofluorescence: use goat anti-mouse IgG-FITC: sc-2010 (dilution range: 1:100-1:400) or goat anti-mouse IgG-TR: sc-2781 (dilution range: 1:100-1:400) with UltraCruz™ Mounting Medium: sc-24941.

RT-PCR REAGENTS

Semi-quantitative RT-PCR may be performed to monitor α -Adaptin 2 gene expression knockdown using RT-PCR Primer: α -Adaptin 2 (h)-PR: sc-43505-PR (20 μ l, 498 bp). Annealing temperature for the primers should be $55-60^\circ\text{C}$ and the extension temperature should be $68-72^\circ\text{C}$.

BIOSAFETY

Lentiviral particles can be employed in standard Biosafety Level 2 tissue culture facilities (and should be treated with the same level of caution as with any other potentially infectious reagent). Lentiviral particles are replication-incompetent and are designed to self-inactivate after transduction and integration of shRNA constructs into genomic DNA of target cells.

RESEARCH USE

The purchase of this product conveys to the buyer the nontransferable right to use the purchased amount of the product and all replicates and derivatives for research purposes conducted by the buyer in his laboratory only (whether the buyer is an academic or for-profit entity). The buyer cannot sell or otherwise transfer (a) this product (b) its components or (c) materials made using this product or its components to a third party, or otherwise use this product or its components or materials made using this product or its components for Commercial Purposes.