SANTA CRUZ BIOTECHNOLOGY, INC.

Centaurin γ3 siRNA (m): sc-60356

BACKGROUND

ADP-ribosylation factor (ARF) family of small GTP-binding proteins are involved in vesicular transport regulation and in controlling cytoskeletal organization and cell adhesion. These proteins are best characterized as regulators of membrane traffic. The Centaurin GTPase-activating protein family comprise a subset of ARF regulatory molecules that transduce PI 3-kinase activation into coordinated control of ARF-dependent pathways. This family includes ASAP1, ACAP1, ACAP2, AGAP1, ARAP1, ARAP2, Centaurin α1 and Centaurin γ3. The Centaurin α1 protein is a high affinity PtdIns(3,4,5)P_3 binding protein enriched in brain. By acting as a GTPase activating protein for ADP ribosylation factor 6 (ARF6), Centaurin α1 is able to switch off ARF6 and inhibit its ability to mediate β_2-adrenoceptor internalization and negatively regulate ARF6 activity by functioning as an *in vivo* PIP_3 dependent ARF6 GAP.

REFERENCES

1. Hawadle, M.A., et al. 2002. Cytohesins and centaurins control subcellular trafficking of macromolecular signaling complexes: regulation by phosphoinositides and ADP-ribosylation factors. Biol. Res. 35: 247-265.
2. Thacker, E., et al. 2004. The ARF6 GAP Centaurin α1 is a neuronal Actin-binding protein which also functions via GAP-independent activity to regulate the Actin cytoskeleton. Eur. J. Cell Biol. 83: 541-554.
3. Venkateswarlu, K., et al. 2004. Centaurin-α1 is an *in vivo* phosphatidylinositol 3,4,5-trisphosphate-dependent GTPase-activating protein for ARF6 that is involved in Actin cytoskeleton organization. J. Biol. Chem. 279: 6205-6208.
4. Venkateswarlu, K., et al. 2005. Centaurin-α1 interacts directly with kinesin motor protein KIF13B. J. Cell Sci. 118: 2471-2484.
5. Lawrence, J., et al. 2005. Centaurin-α1, an ADP-ribosylation factor 6 GTPase activating protein, inhibits β_2-adrenoceptor internalization. Mol. Pharmacol. 67: 1822-1828.
6. Kanamarlapudi, V. 2005. Centaurin-α1 and KIF13B kinesin motor protein interaction in ARF6 signalling. Biochem. Soc. Trans. 33: 1279-1281.
7. Hayashi, H., et al. 2006. Centaurin-α1 is a phosphatidylinositol 3-kinase-dependent ac ERK1/2 mitogen-activated protein kinases. J. Biol. Chem. 281: 1332-1337.

CHROMOSOMAL LOCATION

Genetic locus: Agap3 (mouse) mapping to 5 A3.

PRODUCT

Centaurin γ3 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 µM solution once resuspended using protocol below. Suitable for 50-100 transfections. Also see Centaurin γ3 shRNA Plasmid (m): sc-60356-SH and Centaurin γ3 shRNA (m) Lentiviral Particles: sc-60356-V as alternate gene silencing products.

For independent verification of Centaurin γ3 (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-60356A, sc-60356B and sc-60356C.

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 µl of the RNAse-free water provided. Resuspension of the siRNA duplex in 330 µl of RNAse-free water makes a 10 µM solution in a 10 µM Tris-HCl, pH 8.0, 20 mM NaCl, 1 mM EDTA buffered solution.

APPLICATIONS

Centaurin γ3 siRNA (m) is recommended for the inhibition of Centaurin γ3 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 µM in 66 µl. 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

Centaurin γ3 (E-8): sc-271793 is recommended as a control antibody for monitoring of Centaurin γ3 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-IgGκ BP-HRP: sc-516102 or m-IgGκ 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-IgGκ BP-FITC: sc-516140 or m-IgGκ 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 Centaurin γ3 gene expression knockdown using RT-PCR Primer: Centaurin γ3 (m)-PR: sc-60356-PR (20 µl). 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.