

# Centaurin $\gamma$ 3 siRNA (h): sc-60355

## 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  $\alpha$ 1 and Centaurin  $\gamma$ 3. The Centaurin  $\alpha$ 1 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  $\alpha$ 1 is able to switch off ARF6 and inhibit its ability to mediate  $\beta_2$ -adrenoceptor internalization and negatively regulate ARF6 activity by functioning as an *in vivo* PIP<sub>3</sub> dependent ARF6 GAP.

## REFERENCES

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2. Thacker, E., et al. 2004. The ARF6 GAP Centaurin  $\alpha$ 1 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- $\alpha$ 1 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- $\alpha$ 1 interacts directly with kinesin motor protein KIF13B. *J. Cell Sci.* 118: 2471-2484.
5. Lawrence, J., et al. 2005. Centaurin- $\alpha$ 1, an ADP-ribosylation factor 6 GTPase activating protein, inhibits  $\beta_2$ -adrenoceptor internalization. *Mol. Pharmacol.* 67: 1822-1828.
6. Kanamarlapudi, V. 2005. Centaurin- $\alpha$ 1 and KIF13B kinesin motor protein interaction in ARF6 signalling. *Biochem. Soc. Trans.* 33: 1279-1281.
7. Hayashi, H., et al. 2006. Centaurin- $\alpha$ 1 is a phosphatidylinositol 3-kinase-dependent ac ERK1/2 mitogen-activated protein kinases. *J. Biol. Chem.* 281: 1332-1337.

## CHROMOSOMAL LOCATION

Genetic locus: AGAP3 (human) mapping to 7q36.1.

## PRODUCT

Centaurin  $\gamma$ 3 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 Centaurin  $\gamma$ 3 shRNA Plasmid (h): sc-60355-SH and Centaurin  $\gamma$ 3 shRNA (h) Lentiviral Particles: sc-60355-V as alternate gene silencing products.

For independent verification of Centaurin  $\gamma$ 3 (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-60355A, sc-60355B and sc-60355C.

## 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

Centaurin  $\gamma$ 3 siRNA (h) is recommended for the inhibition of Centaurin  $\gamma$ 3 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

Centaurin  $\gamma$ 3 (E-8): sc-271793 is recommended as a control antibody for monitoring of Centaurin  $\gamma$ 3 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 Centaurin  $\gamma$ 3 gene expression knockdown using RT-PCR Primer: Centaurin  $\gamma$ 3 (h)-PR: sc-60355-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.

## PROTOCOLS

See our web site at [www.scbt.com](http://www.scbt.com) for detailed protocols and support products.